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PURINE AND PYRIMIDINE BASES AS GROWTH SUBSTANCES FOR LACTIC ACID BACTERIA

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In 1936 Richardson[1] showed that uracil was essential for the anaerobic growth of *Staphylococcus aureus*, but not for aerobic growth of the same organism. Of five strains tested three required uracil, while one required both guanine and uracil for growth. Thymine or cytosine did not replace uracil for this organism. These experiments suggested that hydrolytic products of nucleic acids might become factors limiting growth of various organisms under certain conditions. Bonner and Haagen-Smit[2] in 1939 showed that adenine greatly stimulated growth of leaves under defined conditions, while Möller[3] showed that adenine was required for growth of *Streptobacterium plantarum*. Pappenheimer and Hottle[4] recently showed that adenine was necessary for the growth of a strain of Group *A* hemolytic streptococci; it could be replaced by hypoxanthine, guanine, xanthine, guanylic acid or adenylic acid. They made the very interesting observation that adenine was unnecessary for growth of this organism if the carbon dioxide tension was maintained at a sufficiently high level.

Snell and Peterson[5] investigated the properties of an unidentified growth factor for *Lactobacillus casei* ϵ, and concluded that it showed some properties in common with purines, although it was not replaceable by any of the known naturally occurring purines. The failure of known purines to support growth does not indicate that they are not required, since they may be required in addition to other unidentified substances. In view of the above results we have investigated the rôle of purine and pyrimidine bases in the growth of lactic acid bacteria, utilizing in particular organisms which appear to be less fastidious in their growth requirements than is *L. casei* ϵ. Results of this investigation show that for various organisms of this group, each of the purine and pyrimidine bases which occur naturally in nucleic acids—guanine, adenine, uracil and thymine—may become the factor limiting growth.

Experimental.—The basal medium used had the following composition:

	AMOUNT PER 100 CC. OF MEDIUM
Acid hydrolyzed casein (*Labco*, vitamin-free)	0.5 g.
Sodium acetate	0.6 g.
Glucose	1.0 g.
Cystine hydrochloride	10 mg.
Tryptophane	10 mg.
Inorganic salts	
KH_2PO_4	50 mg.
K_2HPO_4	50 mg.
$MgSO_4 \cdot 7H_2O$	20 mg.
NaCl	1 mg.
$FeSO_4 \cdot 7H_2O$	1 mg.
$MnSO_4 \cdot 4H_2O$	1 mg.
Riboflavin	10 μg.
Calcium pantothenate	10 μg.
Vitamin B_6	10 μg.
Thiamin	10 μg.
Nicotinic acid	10 μg.
Biotin concentrate	equivalent to 0.01 μg. pure biotin

The biotin concentrate used was prepared essentially by following through the first few steps in Kögl's procedure.[6] It contained 0.2% biotin when assayed by the yeast method[7] against a sample of Kögl's pure biotin methyl ester. Biotin is essential for the growth of several of the lactic acid bacteria on media similar to that above; it was first reported essential for certain of these organisms by Möller;[3] all of the other vitamins listed with the exception of thiamin have been shown to be growth requirements of representative organisms of this group.[3, 5, 8, 9]

Supplements whose effects were to be tested were added in desired amounts to the culture tubes and the total volume adjusted to 5 cc. To these were then added 5 cc. of the above basal medium (prepared in double the given concentrations and adjusted to pH 6.8), the tubes were plugged with cotton, autoclaved at 15 pounds steam pressure for 15 minutes, cooled and inoculated. Inocula were grown for 24 hours at 30° in Medium B supplemented with 1 μg. of calcium pantothenate (Merck) per culture,[10] centrifuged out, resuspended in 0.9% sodium chloride solution and diluted to the desired density for use. Test organisms used were *Lactobacillus arabinosus* 17–5, *Lactobacillus pentosus* 124–2, *Leuconostoc mesenteroides* P-60 and *Streptococcus lactis* R. Extent of growth was measured by comparing turbidities quantitatively in the thermoelectric turbidimeter.[11] This was calibrated by use of a cell suspension of the organism in question.

The effect of supplementing the basal medium with various purine and pyrimidine bases on the growth of *L. arabinosus* is shown in table 1, columns 1–4.

TABLE 1

EFFECT OF ADENINE, GUANINE AND URACIL ON GROWTH OF *Lactobacillus arabinosus*

μG. SUPPLEMENT PER 10 CC. MEDIUM	MG. MOIST CELLS PRODUCED PER CULTURE (10 CC.) ADENINE SULFATE	GUANINE HYDROCHLORIDE	URACIL	URACIL + 100 μG.* ADENINE PER CULTURE	URACIL + 100 μG.† ADENINE PER CULTURE
0	1.2	1.2	1.2	9.0	4.9
0	1.2	1.2	1.2	9.0	4.8
0.3	...	...	...	..	5.4
1.0	2.0	1.9	...	10.0	7.3
3.0	2.3	2.2	1.4	11.0	9.0
10.0	3.7	2.4	1.5	9.7	8.0
30.0	5.2	4.1	1.4	11.0	8.0
100.0	9.0	4.9	1.7	..	...
300.0	9.0	4.8	...	..	...

* Columns 2–5: 24-hr. incubation at 30°. Inoculum 30 μg. moist cells per culture tube.

† Column 6: 18-hr. incubation at 30°. Inoculum 10 μg. moist cells per culture tube.

Adenine has a striking effect on growth; guanine replaces it effectively at low doses, but does not permit growth to proceed to such a high level. Uracil is ineffective when tested alone.

The inoculum in the above experiment was about 30 μg. moist cells per 10 cc. of medium; at the end of 24 hours the control tube (without adenine) contained 1.2 mg. of cells, while the tube with adequate adenine contained 9.0 mg. of cells. Evidently extensive multiplication has occurred even in the absence of added adenine; its rate, however, is limited by the amount of adenine present. Separate experiments showed that the organism could be serially subcultured indefinitely in the base medium without adenine; if growth were allowed to continue for several days, density of growth in the cultures without adenine approached that in cultures containing adenine.

Thus adenine should be considered as a substance limiting the *rate* of growth (stimulatory substance) rather than as a substance limiting the *extent* of growth (essential growth substance) for this particular organism. It is evident, however, that such distinctions are of little importance in so far as the biochemical importance of a compound is concerned. Thus an external supply of biotin greatly increases rate of growth of yeast, but is not essential for growth;[6, 12] it is, however, essential for the growth of certain clostridia[13] and lactic acid bacteria.

The rate at which multiplication must occur to produce visible growth will be increased either by decreasing the amount of inoculum or the incubation period, or both. Under these conditions uracil, though completely ineffective in growth stimulation in the absence of adenine, proved stimulatory to growth in its presence (table 1, columns 4–6). Separate experiments showed that uracil was completely replaced by cytosine* and guanine by xanthine in the growth of *L. arabinosus*. Addition of both

guanine and adenine resulted in only a very slight growth increase over that secured with adenine alone.

Exactly the same relationships between the presence of adenine and uracil and growth proved to hold with *Lactobacillus pentosus* as with *L. arabinosus*. Our results (table 2) showed that increasing the carbon dioxide tension had no effect on the growth of these organisms or on the growth-stimulating properties of adenine, in contrast to its effect on certain hemolytic streptococci.[4] Adenylic acid (yeast) proved to be almost unavailable, but had slight activity at high levels.

TABLE 2

EFFECT OF CARBON DIOXIDE TENSION ON GROWTH REQUIREMENT FOR ADENINE; AVAILABILITY OF YEAST ADENYLIC ACID

	L. arabinosus					*L. pentosus*				
μg. supplement per culture*	0	3.0	10.0	30.0	100.0	0	3.0	10.0	30.0	100.0
Mg. moist cells per culture										
Adenine sulfate (CO_2-free air)	2.3	2.7	3.3	4.8	5.8	1.3	2.0	2.4	3.8	4.2
Adenine sulfate (10 mm. CO_2 tension)	2.0	2.3	2.8	4.2	5.6	1.1	1.8	2.4	3.9	4.9
Adenylic acid (CO_2-free air)	2.3	2.1	2.3	2.4	2.7	1.4	1.4	1.6	1.9	2.2
Adenylic acid (10 mm. CO_2 tension)	2.0	2.0	2.0	2.2	2.7	1.3	1.3	1.4	1.8	2.2

* All tubes were supplemented with 100 μg. uracil. Inoculum 30 μg. moist cells per culture.

Attempts to extend these results to one of the heterofermentative lactic acid bacteria, *Leuconostoc mesenteroides*, showed that in the presence of adenine and uracil only slight growth occurred in the first culture; this failed on subculture. Further experiments (table 3) showed that with this organism, guanine rather than adenine was the principal growth substance involved, while adenine had only a slight effect. Guanine is apparently essential for the growth of this organism; in its presence the organism responds further to additions of uracil, cytosine or thymine, which are interchangeable in their growth effects. This response appears partly in reducing the concentration of guanine at which a certain level of growth is achieved. Xanthine replaces guanine to a considerable extent, but does not bring growth to the same high level.

The fact that thymine and uracil are interchangeable in their growth effect on *L. mesenteroides* suggested that for certain organisms, thymine might prove to be an essential growth substance. This proved to be the case with *Streptococcus lactis*. This organism grew only slightly on the base medium supplemented with guanine, adenine and uracil and growth

TABLE 3

PURINE AND PYRIMIDINE BASES AS GROWTH FACTORS FOR *Leuconostoc mesenteroides*

SUBSTANCE ADDED	μG. PER CULTURE	MG. CELLS PER CULTURE*	SUBSTANCE ADDED	μG. PER CULTURE		MG. CELLS PER CULTURE
None	...	0.05	Adenine	100		3.5
			Guanine	100		
Uracil	100	0.15	Uracil	100		
			Guanine		30	3.9
					100	4.7
Cytosine	100	0.15	Cytosine	100		
			Guanine		30	3.8
					100	4.5
Thymine	100	0.15	Thymine	100		
			Guanine		30	3.7
					100	4.7
Adenine	10	0.05				
	30	0.20				
	100	1.20	Adenine	100		
			Uracil	100		
Guanine	10	1.8	Guanine		30	3.9
	30	2.5			100	4.5
	100	3.4				
Xanthine	10	2.0				
	30	2.7				
	100	2.6				

* 24-Hr. incubation at 30°. Inoculum 30 μg. moist cells per culture. Adenine and guanine were added as the sulfate and hydrochloride, respectively.

failed on subculture; much heavier growth was obtained which could be serially subcultured for several transfers when thymine was added. The experiments are summarized in table 4. Adenine is also required by this organism; a mixture of adenine and thymine produces growth as heavy as that produced by a mixture of adenine, guanine, thymine and uracil. Guanine can partially replace adenine, but growth is not as heavy. Uracil does not replace thymine.

Discussion.—From the above results it is evident that each of the purine and pyrimidine bases of nucleic acids may under certain conditions become the factor limiting growth of certain of the lactic acid bacteria. Thus adenine greatly stimulates growth of *L. arabinosus* and *L. pentosus*, and is essential for growth of *S. lactis*. Uracil greatly stimulates growth of *L. arabinosus*, and is helpful to *L. mesenteroides*. Guanine is essential for growth of the latter organism. Thymine is essential for growth of *S. lactis*.

In general, the naturally occurring amino derivatives of the purine or pyrimidine bases are replaceable by the corresponding oxy-derivative. Thus cytosine and uracil are interchangeable, as are guanine and xanthine. In some cases (but not all) guanine and adenine are interchangeable; such

TABLE 4

EFFECT OF THYMINE, URACIL, ADENINE AND GUANINE ON THE GROWTH OF *Streptococcus lactis*

SUBSTANCE ADDED	μG. PER CULTURE	MG. CELLS PER CULTURE*	SUBSTANCE ADDED	μG. PER CULTURE		MG. CELLS PER CULTURE
None	...	0.1	Guanine	100		1.0
			Uracil	100		
Uracil	100	0.05				
Thymine	100	0.1	Guanine	100		2.0
			Thymine	100		
Adenine	100	1.5	Guanine	100		2.7
			Adenine	100		
Guanine	100	1.1	Thymine	100		
			Uracil	100		
Adenine	100	1.1				
Guanine	100		Adenine†	100		
			Thymine		0	0.05
Adenine	100	1.0			0.3	0.15
Uracil	100				1.0	0.50
					3.0	1.70
Adenine	100	2.7			10.0	2.70
Thymine	100				30.0	3.1
					100.0	3.1

* Incubation time: 16 hrs. at 30°. Inoculum 20 μg. moist cells per culture.

† From a separate experiment to determine the quantitative requirement for thymine. Inoculated from a 24-hr. culture of the organism in a tube of the basal medium supplemented with adenine alone.

an interchange, however, never results in as good growth as does use of the preferred purine.

Thymine has not been previously recognized as essential for growth of any living organism, although Hammet and Lavine[14] reported that this compound accelerated proliferation of *Obelia geniculata*. That it should be required by certain bacteria is not surprising, since thymonucleic acid is a recognized constituent of the nuclei of plant cells[15] while thymine and 5-methyl cytosine have been isolated from the nucleic acid of tubercle bacilli.[16]

Summary.—For growth on media of known composition certain lactic acid bacteria require one or more of the following: adenine, guanine, thymine or uracil. In several cases where continued growth occurs in the absence of an added supply of these compounds, its rate is greatly increased by their presence.

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CONTACT WITH MEASLES

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Our colleague Dr. W. L. Aycock has raised the question as to the frequency with which an individual comes in contact with the virus of measles. Although the question seems impossible to answer with definiteness because of inadequacy of data, a discussion of it is not without interest and importance. One reason for its importance is the widespread belief that, at least for some diseases, immunity develops progressively from repeated contacts with the agent rather than from a single contact. In dealing with the kind of statistical material available to us for the case of measles we have no method of attack which will enable us to test the hypothesis of the progressive development of immunity from repeated contacts.

It will therefore be assumed that when a person comes first in contact with the virus he acquires the disease and thereby becomes immune. The average age at which one has measles according to reports of cases is:

For Massachusetts,[1]	1932–1937, inclusive	7.08 years
For Massachusetts,[1]	1932–1937, "under 20,"	6.72 years
For Providence, R. I.,[2]	1919–1935, "under 22,"	5.81 years

As infants are immune for about half a year, we may deduct 0.5 from these figures and assume 6.5 for all ages or 6.2 for ages under 20, in Massachusetts,

or 5.3 if we use Providence figures, as the number of years on the average required for a susceptible first to come in contact with the virus.

The difference between Massachusetts (6.2) and Providence (5.3) is considerable. Some of this may be due to later attack in the rural parts of Massachusetts, and some to differences in the completeness of reporting at corresponding ages in the two regions. Investigations on the reporting of measles show not only that the reporting is deficient but that it tends to be most deficient at the earliest ages, and deficient by different percentages in different places.[3] For cases found by frequent house-to-house canvass in Hagerstown, Md., Sydenstricker and Hedrich find the average age (less 0.5) as 4.7. For the incidence by age inferred by Collins from a discussion of the fractions of persons at stated ages who were said to have had measles,[4] the corresponding figure is 4.0. These last two results are well below that for Providence, let alone that for Massachusetts.

Considering the variations in reporting we cannot say that the various figures are inconsistent and would incline to the belief that five years may fairly be taken as the average time required for a child, after losing his initial immunity, to come in contact with the virus. Although the frequency with which children come in contact with measles probably varies considerably with age, we may accept this figure of five years as one estimate of the frequency of contact with it.

Another way to approach the question is to consider that if $N(x)$ be the number of contacts persons of age x (measured from the middle of the first year of life) have had on the average, the chance that a person has escaped all contacts is, by the "law of small numbers," $e^{-N(x)}$ and the chance that he has had at least one contact is $1 - e^{-N(x)}$. Under the hypotheses adopted, this is the chance that he is immune and, if $I(x)$ be the fraction of the immunes at age x, we may write

$$I(x) = 1 - e^{-N(x)} \qquad (1)$$

Surveys give the value of I, with tremendous fluctuations to be sure, and further indicate that there is a limiting fraction, which Collins takes to be 0.89, beyond which immunization does not go—meaning here by immunization that a person is said to have had measles. If we should apply (1) at ages $5^1/_2$, $10^1/_2$, $15^1/_2$, $20^1/_2$ (x being less by half a year) using respectively for the number of contacts N the figures 1, 2, 3, 4, which would result from assuming a contact every five years on the average as estimated above, we should obtain the immunities at those ages as 63.2%, 86.5%, 95.0%, 98.2%, respectively. The percentages thus found for ages $5^1/_2$ and $10^1/_2$ are not far from those reported by Collins but those for ages $15^1/_2$ and $20^1/_2$ are well above his.

One way to obtain an estimate of the average number of contacts that have been experienced by persons of age x is to reverse (1) and write

$$N(x) = -\log_e [1 - I(x)]. \qquad (2)$$

To use this expression it is necessary to have a good estimate of $I(x)$. For age $20^1/_2$ the expression would be $N(20) = -\log_e [1 - I(20)]$, and as $I(20)$ is likely to be nearly up to 1.00, the expression for $N(20)$ is very sensitive to variations in the estimate of I; thus for the respective values $I(20) = 0.89, 0.95, 0.98, 0.99$, we find $N = 2.2, 3.0, 3.9, 4.6$ contacts which correspond respectively to one contact in 9, 6.7, 5.1, 4.3 years. The question of how widespread an immunity there is in the population at around age 20 is therefore of great importance for the answer to Dr. Aycock's question.

Dr. Chapin, after reviewing the figures with his accustomed care and conservatism, comes to the conclusion:[5] "It is probable, then, that in England, Canada and the United States over 90 per cent of urban populations contract measles at some time during their lives." Since very few contract it after 20, this amounts to agreeing that Collins's limiting figure of 89 per cent is a minimum for urban populations. We believe that a higher minimum may be set with a fair probability. For the whole group of contacts with primary cases in measles families in Providence at ages 15–21 inclusive we have found an immunity[6] of 97.0% before the entrance of measles into the family, which became only 97.4% afterwards. Even if we include all the primary cases at these ages, the immunity was 95.3% before and 97.5% afterwards. It is impossible to say what is the immunity in Providence as a whole in the age group 15–21 but there seems little reason to believe that the families into which measles did not come were any less immune than those into which it did come, or that the former were any more immune than the latter after the passage of measles. This would mean that we should set 95.3% as a minimum and 97.5% as a maximum in estimating for Providence the immunity in the whole age group 15–21.

It should not be inferred from our first use of (1) combined with the assumption that $N(x) = x/A$, where A is the average age at which one has measles, that we believe N is indeed proportional[7] to x; for, clearly, if we know the values of $I(x)$ we could compute from the form (2) the values of $N(x)$ and, by subtraction of two values computed for two different ages, obtain the number of contacts between those ages. This has been done and the results are given in the table on four different assumptions:[8] (1) Collins's smoothed values of $I(x)$ from his surveys,[4] (2) Sydenstricker and Hedrich's case rates by age as smoothed by us,[9] (3) Massachusetts cases scaled up in an attempt reasonably to allow for different factors of reporting at different ages,[10] (4) Providence case rates scaled up by the constant factor 2.27.

NUMBER OF CONTACTS N TO GIVEN AGE AND DIFFERENCE ΔN

	(1) COLLINS		(2) SYDENSTRICKER		(3) MASSACHUSETTS		(4) PROVIDENCE	
AGE	N	ΔN	N	ΔN	N	ΔN	N	ΔN
2	0.18		0.22		0.15		0.13	
		0.38		0.36		0.27		0.23
4	0.56		0.58		0.42		0.36	
		0.50		0.51		0.46		0.42
6	1.06		1.09		0.88		0.78	
		0.53		0.81		1.09		0.87
8	1.59		1.90		1.97		1.65	
		0.37		0.84		0.98		1.30
10	1.96		2.74		2.95		2.95	
		0.24		2.12		1.25		2.45
15	2.20		4.86	....	4.20		5.40	
		0.01				0.60		1.51
20	2.21		....	....	4.80		6.91	....
		0.00				0.88		
30	2.21		....	....	5.68		....	....

The figures are not consistent, nor are they reliable. However, in the age groups 4 to 6 and 6 to 8, which are generally considered most important as judged by the number of cases reported, the discrepancies in the four sets are not too serious. It would appear that a child between four and six years of age experiences about half a contact, i.e., is coming across the virus of measles at the rate of about once in four or five years. The rate is higher for children between 6 and 8 and is more discrepant; according to Collins it would still be about once in four years, but according to the other three figures it would be nearly as high as once in two years. Under four years of age the rate is lower than from 4 to 6; above the age of ten great inconsistencies appear in the figures obtained from the different assumptions.

To sum up we may state that the unsatisfactory evidence seems to indicate that the rate of contact with the virus of measles rises from a low value of once in six to ten years in the first years of life to a maximum which may be as high as once in two years sometime between the age of six and the age of ten and then falls off, with the result that by the age of twenty a person has had four or five contacts altogether.

These conclusions are based upon the all-or-none hypothesis that the immunity to measles results from a single attack; there seem to remain about two per cent of young adults who do not contract the disease upon intimate exposure and who may have acquired their immunity by a cumulative process, though this is not a necessary inference.[6] The age incidence of some other immunizing diseases of childhood is so similar to that for measles that it is tempting to assume by analogy that for those also the process of immunization is the same; but, again, this inference is not necessary, for it is certainly possible that immunity acquired for some

persons at a definite time by one contact and for other persons gradually over time by many contacts might give rise to an age distribution of cases not very different from that arising on the all-or-none hypothesis.

[1] Computed from the age distributions given in *Annual Report of the Department of Public Health, Massachusetts*, for the years in question. We give total cases and annual rates per thousand population.

AGE	CASES	RATE	AGE	CASES	RATE	AGE	CASES	RATE
<1	2701	7.5	7	19943	48.7	30–35	364	0.21
1	5942	19.1	8	13287	31.6	35–45	337	0.092
2	8093	22.1	9	7385	17.2	45–55	117	0.040
3	9317	24.6	10–15	12352	5.62	55–65	48	0.024
4	11587	30.4	15–20	2169	1.02	>65	34	0.021
5	15749	40.3	20–25	862	0.44	unknown	6334	
6	24087	59.7	25–30	538	0.28			

The unknowns were distributed pro rata over the age groups before computing the rates. Populations were taken from the *Report on the Census of Unemployment in Massachusetts as of January 2, 1934*, except that for the population under 1 births were used.

[2] "Measles and Scarlet Fever in Providence, R. I., 1929–1934 with Respect to Age and Size of Family," *Proc. Amer. Philos. Soc., Philadelphia*, **80** (No. 3), 357–476 (1939). See p. 397.

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[4] "Age Incidence of the Common Communicable Diseases of Children," by S. D. Collins, *U. S. Public Health Reports, Washington*, **44** (No. 14) 763–826 (1929).

[5] "Measles in Providence, R. I., 1858–1923," by C. V. Chapin, *Amer. Jour. Hygiene, Baltimore*, **5**, 635–655 (1925), especially p. 643.

[6] Here again immunity means those who are said to have had measles; the figures obtained by follow-up work in measles families are higher than those found on general surveys, as is perhaps natural from "psychological" reasons. We have an unpublished series of 5623 measles families in Providence (1935–1939) in which there are 5512 mothers reported as contacts (parents of at least one child 21 or under who was a primary case); of these, 327 did not know whether they had had measles, 5068 had had measles and 117 had not; yet of these 117 putative susceptibles only 4 developed the disease. This presumably should be interpreted as indicating that most of the 113 "susceptibles" who escaped were actually immune in the sense that they would not contract the disease under exposure to intimate contact. It would be interesting, if it were practicable, to do a series of tests on young adults who were supposed never to have had measles and who nonetheless did not contract it upon close exposure to determine whether they were actively immune. It may be noted that the 117 mothers who had not had measles formed about two per cent of the whole series of 5623. It is known that, according to the time of administration, measles may be prevented, leaving the child susceptible, or modified, with the acquirement of immunity, by administration of immune serum or placental extract and it is possible that children exposed to measles during that period in their first year of life in which their initial immunity is wearing off may have their measles so modified as not to be recognized under ordinary conditions and yet have it sufficiently to give an

immunity. In Providence we found over a six-year period 334 contacts during the first year of life who escaped; this is an average of 57 or about 1.4% of the annual births; how many more there were who were unreported is impossible to estimate. It is perhaps not out of the question that something approaching 2% of the population becomes immunized by having a non-recognized case. If a study were made of the young adults who had not had measles but did not take it on close exposure, it would be well to enquire particularly about their history of previous exposure within the family.

[7] The law $I = 1 - e^{-x/A}$ is particularly simple. It has the property that if we take the case rate as $C(x) = dI/dx$, the average length of time persons immune at age x take to contract the disease is A, when deaths are neglected, as they may be to a first approximation. But even if this law applied in the case of groups homogeneous with respect to exposure, it could not apply to an inhomogeneous group made up of fractions $p_1, p_2, \ldots$ of the population with different mean frequencies of exposure $1/A_1, 1/A_2, \ldots$ because

$$I(x) = 1 - p_1e^{-x/A_1} - p_2e^{-x/A_2} - \ldots, p_1 + p_2 + \ldots = 1$$

cannot be reduced to the form $1 - e^{-x/A}$, and there is reason to believe that the population is actually decidedly inhomogeneous with respect to rate of exposure.

[8] The general theory of our problem is a special case of Ronald Ross's "Application of the Theory of Probabilities to the Study of *a priori* Pathometry," *Proc. Roy. Soc., London*, **A 92**, 204–230 (1916). For our case a simple derivation of the relations may be given. If P be a population and I the fraction of immunes, the number of immunes is IP; if C is the case rate (per capita) and f the case fatality (per capita) of a disease which immunizes permanently, the number who in time dt contract the disease is $CPdt$ and the survivors are $(1 - f)CPdt$ who represent an increase of immunes; if D' be the death rate of immunes from all causes, their losses will be $D'IPdt$. Hence

$$d(IP) = (1 - f)CPdt - D'IPdt$$

But $d(IP) = IdP + PdI$ and $dP = DPdt$ where D is the death rate of the whole population from all causes. Hence

$$dI/dt = (1 - f)C + I(D - D').$$

A number of tacit assumptions have been made, such as that the disease is of short duration, which seems fair enough for measles, and that immigration into and emigration from the population may be neglected. If it be assumed that the general death rate and that on immunes differ only by the death rate fC due to the disease and if it be further noted than an increase of time is equivalent to an increase of age x, we may write

$$dI/dx = C - f(1 - I)C.$$

Considering the inaccuracies of reporting and the small values of the case fatality for measles, we have decided that we may safely use $dI/dx = C$ or even $\Delta I = C\Delta x$ for finite age intervals provided C be taken as the average case rate during the age interval. It may further be observed that the attack rate $a(x)$ upon susceptibles is

$$a = \frac{C}{1 - I} = \frac{1}{1 - I}\frac{dI}{dx} = \frac{d}{dx}[-\log_e(1 - I)] = \frac{dN}{dx}.$$

[9] We used $I = 3.49\,[1 - e^{-.0383x}]$ for $x < 6.3$ and $I = 1 - 3.57e^{-.423x}$ for $x > 6.3$. This gives figures for $C(x)$ proportional to those of Sydenstricker and Hedrich[3] after allowance for the immunity in early life; we had to introduce a factor of propor-

tionality because their case rates were apparently taken during an epidemic so severe as to imply more than 100% immunization! Their data is not applicable after age 15.

[10] We have no very good basis for assigning the factors which we used for multiplying the number of cases for under-reporting, viz.,

age	<1	1	2	3	4	5	6	7	8	9	10+
factor	5.65	5.15	4.65	4.15	3.66	3.16	2.66	2.33	1.99	1.66	1.33

*THE TWELFTH PRIMARY TRISOMIC TYPE IN NICOTIANA SYLVESTRIS**

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For over ten years the cytogenetics of the trisomic condition in *Nicotiana sylvestris* ($n = 12$) has been under investigation in the University of California Botanical Garden. In a detailed report upon a portion of the results (Goodspeed and Avery, 1939) it was indicated that the extended series of aneuploid and other chromosomal variants under discussion had been derived from a number of different sources (loc. cit., p. 383). The report contained descriptions of the morphological distinctions from one another and from diploid which were displayed by the eleven primary trisomic types which had up to that time been identified, together with evidence concerning the origins of other trisomic types, etc. It is now possible to report the occurrence and identification of the twelfth primary trisomic of *Nicotiana sylvestris*.

In progeny of $3n - 1 \times 2n$ a double trisomic appeared which in external morphology showed primarily characters peculiar to the primary trisomic Puckered (loc. cit., p. 396). The alterations in Puckered morphology which this plant exhibited could not be assigned to combination with any one of the other identified trisomic types. From this plant crossed with $2n$ ♂ a progeny was produced which consisted of 24 diploid, 14 Puckered, 12 plants of a hitherto unknown trisomic type and 3 double trisomics which in external morphology represented a combination of Puckered and the new trisomic type.

This new, twelfth primary trisomic called "Stiff" has appeared a second time and from a different source. The primary trisomic Inflated (loc. cit., p. 399) in the asynaptic condition was crossed with diploid ♂ and in a progeny of 25 plants one trisomic Stiff occurred. This plant crossed with diploid ♂ gave 5 Stiff and 20 diploid. From one of these five plants crossed with diploid ♂ a progeny of 50 plants was grown of which 19 were Stiff

and 31 diploid, showing in this case 38% transmission of Stiff through eggs (loc. cit., p. 415, table 13).

When contrasted in morphological characters with diploid *Nicotiana sylvestris* the trisomic Stiff is characterized by shorter stature; strongly developed laterals; basal leaves stiff, horizontal or horizontal-erect, ovate-lanceolate with wide wing on the petiole which is extended into broad auricles; leaves dark grayish green, midrib greenish and sunken, veins sometimes irregular and often in twos, margins broadly waved, only slightly pubescent, small necrotic areas along leaf margins between veins, in age extending to give entire leaf a "sunburned" appearance; flower pendent, limb longer and narrower (91 × 32 mm.), tube narrower with infundibulum pronounced but not inflated, corolla lobes incised but broad at the base and early reflexed, calyx inflated below.

The extreme stiffness of the leaves, their necrotic areas and the length of the flower, which is exceeded only by that of Enlarged (loc. cit., p. 393) sets Stiff apart morphologically from all the other eleven trisomic types of *Nicotiana sylvestris* as well as from the diploid. Among the other primary trisomics, it most closely resembles Bent (loc. cit., p. 397) from which it is readily distinguished by the prominent morphological characters just referred to.

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† Dr. Avery's death occurred during the course of this investigation. Acknowledgment is made of assistance by Dr. M. V. Bradley in carrying it forward.

Goodspeed, T. H., and Avery, P., "Trisomic and Other Types in *Nicotiana sylvestris*," *Jour. Gen.*, **38** (3), 381–458 (Sept., 1939).

ISOLATING MECHANISMS IN TREE FROGS

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Two small early-breeding tree frogs are found at Bloomington, Indiana *Hyla crucifer* and *Pseudacris triseriata* differ in a number of morphological characters, have different mating calls and to a certain extent utilize different breeding sites. In view, however, of the fact that the two species frequently breed in the same ponds, and that large series of specimens sometimes contain a few puzzling individuals, it has seemed desirable to investigate the possibility of interbreeding between the two species.

Examination of a series of 426 males and 80 females from Bloomington shows that the toe pads of *P. triseriata* rarely attain one millimeter in diameter while those of *H. crucifer* are rarely as small as one millimeter. The dorsal pattern of *H. crucifer* is usually an X while that of *P. triseriata* usually consists of three longitudinal stripes or rows of dots. *Hyla crucifer* is frequently reddish in color while *P. triseriata* is never so colored. The fourth toe of the hind foot of *P. triseriata* is proportionately longer than the corresponding toe of *H. crucifer*. It is apparent that the two species differ in many respects morphologically.

The eggs of *H. crucifer* are laid singly and have a thin, sharply-defined jelly envelope; those of *P. triseriata* are laid in a mass and each egg has a thick, poorly-defined jelly envelope. The breeding call of *H. crucifer* is a shrill peep while that of *P. triseriata* may be likened to the sound produced by drawing one's fingernail across the teeth of a comb.

The two species may breed in the same pond at the same time but this is not an invariable rule as the following collecting data show:

LOCALITY	DATE	*H. crucifer* ♀	*H. crucifer* ♂	*P. triseriata* ♀	*P. triseriata* ♂
Bloomington 4 N.	3/9/38	0	4	15	46
Bloomington 4 N.	3/13/39	0	10	8	33
Bloomington 2 N.	3/17/39	4	39	1	5
Bloomington 4 N.	3/18/38	6	32	2	15
Bloomington 4 N.	3/22/39	0	1	2	42
Bloomington 4 N.	3/22/40	0	27	0	4
Bloomington 2 N.	3/23/39	1	20	0	0
Bloomington 4 N.	4/22/39	7	23	6	38
Bloomington 4 N.	4/27/40	1	30	8	28

Within a given pond there may be aggregations consisting mainly of one species at various points. Such aggregations are probably due to the response of frogs to the call of their own species.

Male *H. crucifer* readily clasp female *P. triseriata*. Although quantitative and critical data are lacking, observations seem to indicate that male *P. triseriata* show a greater degree of sexual isolation. In 39 instances females laid eggs while clasped by a male of another species. The history of the crosses may be summarized:

NO.	CROSS	SOME CLEAVAGE IN:	TADPOLES	METAMORPHOSIS
17	♀ *H. crucifer* × ♂ *P. triseriata*	2 crosses	6	0
22	♀ *P. triseriata* × ♂ *H. crucifer*	19 crosses	1123	43

Non-hybrid matings showed almost perfect cleavage and subsequent development. Of the two crosses of ♀ *H. crucifer* × ♂ *P. triseriata* in which cleavage was recorded, one involved a single egg and the other not more

than 3% of the egg mass. None of the six tadpoles produced lived longer than 17 days and all were abnormal in body shape. The reciprocal cross was more successful. Of the 22 crosses, 19 showed cleavage; in most cases the percentage of eggs cleaving was high (80–100%). While developmental abnormalities were found from early cleavage to metamorphosis, the greatest number of failures took place during gastrulation. On the average about 10% of a given batch of eggs reached the tadpole stage, but many of these were unable to swim or feed and soon died without growing appreciably. Some tadpoles grew quite normally for a time but eventually

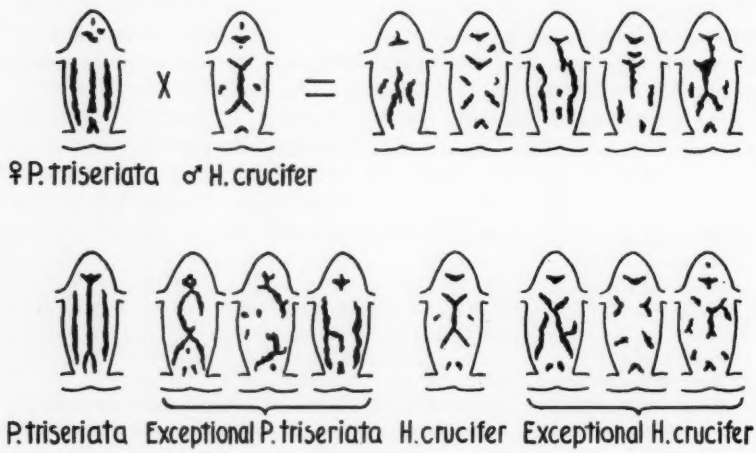

FIGURE 1

A typical cross is shown in the first horizontal row. The second horizontal row shows typical and exceptional markings for *H. crucifer* and *P. triseriata;* specimens with exceptional markings were classified on the basis of characters other than dorsal markings.

acquired an emaciated appearance similar to that of hyperthyroid tadpoles and died without metamorphosing. The larval period of the 43 apparently normal tadpoles which metamorphosed varied from 71 to 186 days. None of the hybrids was reared to adult size but lack of viability can scarcely be attributed to their hybrid nature since non-hybrid animals being reared at the same time failed to survive longer than the hybrids. Of the three hybrids which survived longer than one year, one lived 424 days (272 days beyond metamorphosis), one 371 days (195 days beyond metamorphosis), and one 380 days (259 days beyond metamorphosis).

The absence of cleavage in the great majority of *H. crucifer* eggs may be due to lack of penetration of the *P. triseriata* sperm. Tchou Su[2] cites a

number of cases among Chinese anurans in which sperm of one species penetrate the egg of a second species but the sperm of the second species fail to penetrate the egg of the first species.

The toe pads of the hybrids were more like those of *P. triseriata* than those of *H. crucifer*. Dorsal markings were more or less intermediate. A typical cross is shown in figure 1; in addition to the parents and five offspring, several atypically marked *H. crucifer* and *P. triseriata* from field collections are shown. Such atypically marked animals, which comprise perhaps 1–2% of the total population while the great majority of animals are marked with an X (*H. crucifer*) or with stripes or rows of dots (*P. triseriata*), can usually be readily classified on the basis of characters other than dorsal markings. It is evident that they resemble the hybrids as far as dorsal markings are concerned.

It is well known that the markings of young animals frequently differ from those of adults. Since the determinations on hybrid dorsal markings were made on young animals it seemed necessary to check on the markings of non-hybrid immature animals. This was done both on laboratory reared and field collection animals and the variation in dorsal pattern of a series of newly-metamorphosed animals was found to follow closely that of a series of adults. These data, incidentally, render unlikely any supposition that the hybrids are parthenogenetic instead of true hybrids.

The available evidence is insufficient to say that occasional hybridization between *H. crucifer* and *P. triseriata* does or does not take place. If such hybridization does occur, there is still the question of hybrid sterility or fertility. At any rate, since the viability of hybrid offspring is so limited, it is apparent that occasional hybridization would do little to alter the distinctness of the two species.

[1] Fellow of the National Research Council, 1940–41. The greater part of the work on which this report is based was carried on at Indiana University, 1937–40.

[2] Tchou Su, "L'hybridation chez les anoures de Canton (Chine)," *C. R. Acad. Sci. Paris*, **202**, 242–244 (1936).

THE TIME INTERVAL BETWEEN X-RADIATION OF SPERM OF DROSOPHILA AND CHROMOSOME RECOMBINATION

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The Nature of the Problem.—The present article records the results of an experiment in which comparison has been made between the frequencies of detectable chromosome breaks produced by two equivalent doses of x-rays, the one administered continuously, the other in a series of fractions. This approach to an understanding of the mechanism involved in the production of induced chromosomal rearrangements stems from the study of Bauer, Demerec and Kaufmann,[1] in which the effects of x-rays on the sperm of *Drosophila melanogaster* were measured by analysis of changes in the salivary gland chromosomes. By comparison of break frequencies at different dosages an effort was made to determine whether breakage and recombination are part of a single process and occur simultaneously (the hypothesis of Serebrovsky), or whether the individual breaks participating in a rearrangement are produced independently. In the latter case it seemed probable that an appreciable interval should elapse between the time of induction of breaks and their recombination (the hypothesis of Stadler). In the event that a contact mechanism was involved, there should exist on theoretical grounds a linear proportionality between dosage and the frequency of chromosome rearrangements, whereas, if each break were produced independently, the frequency should increase as the square of the dosage. The results of this study showed that break production did not increase linearly with the dosage in the span between 1000 and 5000 roentgens. Further analysis of these data[2] showed that the relationship of dosage to break frequency approximated a 1.5 power curve, or possibly a 2 power curve at the lower levels, although deviating widely from either at the 3000-r point. In an independent study of the same problem, Catcheside[3] obtained results which he interpreted as favoring the concept of linear proportionality.

Because of these apparently contradictory results it seemed best to approach the question of delayed attachment by comparison of the effects of a given dose of irradiation when applied continuously with those produced by an equivalent dose given intermittently. It appeared possible that the sperms receiving the discontinuous treatment should furnish a smaller number of detectable breaks than those treated uninterruptedly, if in the interval between successive treatments any "healing" should occur to restore the original gene sequence, so that such broken ends would not be available to participate with those subsequently produced in the

formation of chromosomal aberrations. Moreover, the data of Bauer, Demerec and Kaufmann[1] suggested that the frequency of the more complex rearrangements increased with the dosage. It seemed, therefore, that if any appreciable amount of recombination were occurring in the interval between successive irradiations, the sperms receiving the treatment in fractions should give a preponderance of the simpler rearrangements and a dearth of the complex ones in which numerous broken ends are involved in a single aberration. A further possibility was suggested by the discovery of Dobzhansky and Sturtevant[4] that the overlapping and included types of inversions which occur in natural populations of *D. pseudoöbscura* arise through two successive inversion processes. Since inversions, each involving two breaks, are induced relatively frequently at the lower dosage levels (1000 and 2000 r) it was thought that the production of such changes during the earlier fractions of a discontinuous-treatment series should increase the possibilities for superposition of other inversions during the subsequent fractions with the formation thereby of the overlapping and included types.

The Methods.—Males of the Oregon-R stock of *D. melanogaster* were irradiated at 3000 roentgens in three fractions of 1000 r each separated by 24-hour intervals, other males at 4000 r in four 1000-r doses spaced at 24 hours, and a third group of males at 4000 r in two 2000-r fractions with a 16-day interval. After completing the final treatment the males were mated with Oregon-R females. Males were discarded at the end of a week during which transfers to fresh food were made on every second day. The F_1 larvae secured from these matings provided the salivary gland chromosomes used in the cytological analysis.

Flies receiving the continuous treatment were irradiated on the same day that the comparable discontinuous-treatment series was begun. They were then stored so that they could be mated at the same time as the animals which had received the equivalent dose in fractions. Analysis of these controls soon showed, however, that they paralleled both in frequency of altered sperms and of chromosome breaks the more extensive material studied in the earlier experiment, namely, that portion of the data of Bauer, Demerec and Kaufmann which had also been obtained by using Oregon-R males. These data are accordingly presented here for comparison with those from the fraction treatments, as are also the extensive data for the 3000-r dose reported more recently by Bauer.[5]

The Results.—In table 1 are summarized the findings showing the percentages of altered sperms and breaks per total sperm for the continuous and intermittent treatments. No differences in frequencies of breaks produced by these two methods are apparent beyond the limits of the experimental errors. The different groups of glands analyzed at each dosage level are, in general, quite homogeneous, even for relatively small samples,

as is shown by comparing the results produced by four fractions of 1000 r with those obtained following a single treatment of 4000 r. In the material receiving two fractions of 2000 r, the first half of the glands analyzed gave essentially the same frequency of breaks as the second half. Occasionally it happens, however, that certain treatments give results which show wide departures from the mean. One 4000-r dosage of two fractions had among 68 glands analyzed only 17.6 per cent altered, and only 63.2 per cent breaks. The controls totaled 48 glands, of which only 14.6 per cent were altered, and there were only 45.8 per cent breaks. The reasons for these reduced values could not be determined, and the data have not been included in the preparation of table 1.

TABLE 1

COMPARISON OF FREQUENCY OF INDUCED CHROMOSOME BREAKS FOLLOWING CONTINUOUS AND FRACTIONATED X-RAY TREATMENT OF MALES

Frequency was determined by salivary chromosome analysis. (No errors are furnished for the percentage of breaks, since the distribution of the number of breaks is not given by a Poisson series.) 1×3000 r; 1×4000 r refer to continuous treatment.

DOSE IN R	TOTAL GLANDS	NO. WITH CHROMOSOMAL ABERRATIONS	PERCENTAGE ALTERED SPERMS	NO. OF BREAKS	PERCENTAGE BREAKS PER TOTAL SPERM
3000					
1×3000*	595	112	18.82 ± 1.60	293	49.24
1×3000†	132	28	21.21 ± 3.56		
3×1000 at 24-hr. intervals	184	43	23.37 ± 3.12	96	52.17
4000					
1×4000†	271	79	29.15 ± 2.76	227	83.76
4×1000 at 24-hr. intervals	44	15	34.09 ± 7.15	36	81.82
2×2000 at 16-day interval	140	39	27.86 ± 3.79	117	83.57
Total fraction treatment	184	54	29.35 ± 3.36	153	83.15

* Data from Bauer, 1939.

† Includes data from Bauer, Demerec and Kaufmann, 1938.

Table 2 shows the frequency of the different types of rearrangements. The number of breaks per sperm are here classified, using the method of Bauer,[5] according to the number of independent rearrangements which they produce. Thus, four breaks may recombine in twos to form inversions or translocations, or all four may be involved in a single complex rearrangement.

In preparing table 2, recourse has again been made to the extensive data provided by Bauer. These data suggest, although it was not apparent in the analysis of Bauer, Demerec and Kaufmann, that the percentage of the total breaks which are involved in the complex type of rearrangement

having 4 or more breaks, is about the same (7–10 per cent) at all the dosage levels here considered. The reality of this type of distribution remains to be determined. Comparison of the effects of the intermittent and the continuous-treatment material is restricted, therefore, by the small number of breaks secured at the lower dosage levels. On the other hand, the existence of the 5- or 6-break complex rearrangements, such as have been found following the 4000-r intermittent treatment, suggests that no recombination occurs between successive fractions. For, if the latter did occur we should expect each of the single complex rearrrangements to be re-

TABLE 2

TYPES OF REARRANGEMENTS PRODUCED FOLLOWING CONTINUOUS AND FRACTIONATED X-RAY TREATMENT OF SPERM OF D. MELANOGASTER

DOSE IN ROENTGENS	TOTAL BREAKS	TOTAL SPERMS	NO. OF INDEPENDENT REARRANGEMENTS	BREAKS PER SPERM 2	3	4	5	6	7	8	9	10	11
*1000 (1 × 1000)	34	616	1	12	2	1	..	..	..	..	..	..	..
*2000 (1 × 2000)	155	654	1	47	8	..	..	..	..	..	..	..	..
			2	..	..	8	1	..	..	..	..	..	..
*3000 (1 × 3000)	293	595	1	74	14	6	..	..	..	..	..	..	..
			2	..	..	13	3	2	..	..	..	..	..
3000 (3 × 1000)	96	184	1	36	4	2	..	..	..	..	..	..	..
(24-hr. interval)			2	..	..	1	..	..	..	..	..	..	..
*4000 (1 × 4000)	427	447	1	86	17	6	..	1	..	..	..	..	..
			2	..	..	21	3	4	..	..	1	..	..
			3	..	..	..	..	..	3	..	..	1	1
4000 (1 × 4000)	49	60	1	10	2	2	..	1	..	..	..	..	..
			2	..	..	1	1	..	..	..	..	..	..
4000 (4 × 1000)	36	44	1	12	..	1	..	..	..	..	..	..	..
(24-hr. interval)			2	..	..	2	..	..	..	..	..	..	..
4000 (2 × 2000)	117	140	1	27	3	..	1	1	..	..	..	..	..
(16-day interval)			2	..	..	3	..	..	..	..	..	..	..
			3	..	..	..	..	..	1	3	..	..	..

*Data from Bauer, 1939.

placed by two or three of the independent 2- or 3-break types which are characteristic of the 1000- and 2000-roentgen doses. In the specific case in which twelve broken ends (6 breaks) participate in a single rearrangement, it seems more likely that some of the breaks were produced during each fraction of the treatment and failed to combine in the intervening period, rather than that all six were produced during the last 2000-r fraction, for the reason that no such complex rearrangement has so far been found in the 2000-r control material. Further information on this matter must await the compilation of more adequate data concerning the types of rearrangements produced at different doses.

No overlapping or included inversions were found following the fraction

treatment. This evidence further strengthens the contention that no recombination occurs between successive treatments.

Discussion.—The close correspondence between the material receiving continuous treatment and that receiving intermittent treatment with respect to the proportions of altered sperms and of chromosomal breaks suggest that no appreciable "healing" occurs in the interval between successive treatments even when they are separated as widely as 16 days. Muller and Makhijani (reported by Muller[6]) found that treatment of impregnated females of *D. melanogaster* in four fractions at weekly intervals, during which the flies were kept at 8°C., gave essentially the same translocation frequency between the second and third chromosomes as did the continuous treatment. The genetic method used for detecting the translocations, although leading to the same conclusion concerning delayed reattachment as does the present study, measures only a portion of the altered sperms, whereas the cytological analysis of salivary chromosomes offers the added criterion of total break production for comparing the experimental materials.

The concurrence of these two independent analyses using different techniques clarifies the confusion that attended earlier work on this problem. In his Woods Hole lecture of 1938, Muller[7] reported that the frequency of chromosomal alterations in Drosophila, measured by genetic methods, increases according to the 3/2 power of the dosage. It has been indicated previously that the data of Bauer, Demerec and Kaufmann seemed, in general, to conform to this value. When Sax,[8] in addition, reported that a similar curve had been obtained for x-ray induced chromosomal breaks in Tradescantia, it seemed that some general principle applicable to the relationship of dosage to break frequency might be involved. But shortly thereafter, Sax[9] showed that the 1.5 value resulted because an appreciable amount of "healing" occurred during the longer periods necessary to produce the higher doses of the series, and that this factor could be eliminated and a curve obtained approaching the theoretical 2-power value if all treatments of a series were delivered synchronously by varying the intensity. On these grounds it was conceivable, as Sax[10] pointed out, that the presumed 1.5 value in Drosophila might likewise result from a certain amount of restitution of original sequences following breakage at the higher dosage levels, since the treatment of Drosophila males had been given at a constant intensity by varying the time. Bauer,[5] however, in his extensive and refined study demonstrated that the total break production at different dosages fits extremely well a 2-power curve, and this finding in itself indicated that the first-formed breaks at higher doses were not being eliminated by "healing" during the progress of the treatment. The lack of an effect of the time-intensity factor was likewise described by Catcheside in a paper presented to the International Congress of Genetics in Edin-

burgh in 1939, in which it was reported that varying the time of treatment from 15 to 300 minutes gave no differences in the frequency of gross chromosomal rearrangements in *D. melanogaster*. More recently, Muller[6] has reported that 2000 roentgens delivered at a rate of 0.05 r per minute gave essentially the same translocation frequency as at 250 r per minute. In the present study it has been shown that no appreciable "healing" or recombination occurs within a 16-day period.

What, then, is the extent of the time during which the breaks or potential breaks induced by irradiating the sperm retain their capacity for recombination? On several grounds it seems probable that recombination is not effected until the sperm nucleus has penetrated the egg. Sidky[11] reports that a translocation has been obtained between an induced break of paternal origin in the third chromosome of *D. melanogaster* and a spontaneous break in a Y-chromosome transmitted through the mother. However, in this case the possibility is not removed that both chromosomes were of paternal origin. Glass[12] did not find any translocations between chromosomes of paternal and maternal origin following irradiation of impregnated females. Such translocations would furnish critical evidence on the question of delayed reattachment. Helfer[13] has observed mosaic salivary glands of *D. pseudoöbscura*, one of which shows four different types of cells with respect to chromosomal rearrangements. A plausible explanation of their origin is that the recombination of broken ends was delayed until after the second cleavage mitosis. Such evidence, however, is at best suggestive, since other plausible explanations of such mosaics have been furnished.

Cytological observations lend some weight to the belief that reattachment may be delayed at least until the time of fertilization. The sperm head of Drosophila is elongated, about as wide as the mitotic metaphase chromosome. Because of its strong basophilic properties, it presents in fixed and stained material an appearance of homogeneity that masks any details of internal organization. It seems probable, however, from observations on sperms of another Diptera[14] that the chromonemata are elongated, closely appressed and therefore immobile. Only after the sperm has penetrated the egg and rounded up to form the male pronucleus do the individual chromosomes become recognizable cytologically. The changes involved in the transition from the conical sperm head to the spherical pronucleus may provide the first opportunity for recombination of chromosome fragments. But if exchanges are possible between chromosome fragments of maternal and paternal origin, recombination must occur subsequent to the formation of the first cleavage spindle upon which the chromosomes of male and female pronuclei remain separated as independent aggregates (gonomery). It thus seems possible that if the propinquity necessary for chromosome rearrangement is not realized within

the male pronucleus, the breaks or potential breaks may remain capable of reunion for a limited time during which contacts with other chromosomes may be realized.

For the present the question must remain open concerning the nature of the physical and chemical processes which permit breaks or potential breaks to retain their capacity for reunion over a period of several weeks.

Summary.—Comparison has been made, using salivary gland chromosomes, of the numbers of breaks induced by irradiating sperm of *D. melanogaster* with two equivalent doses of x-radiation, one of which was delivered continuously, the other in a series of fractions. Comparable frequencies obtained with both types of treatment indicate that no appreciable "healing" occurs even when the interval between successive fractions is 16 days. The presence of multiple-break, complex rearrangements following the fractionated treatment strengthens the evidence for the theory that breakage precedes reunion.

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MECHANISM OF THE ORIGIN OF X-RAY INDUCED NOTCH DEFICIENCIES IN DROSOPHILA MELANOGASTER

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It is well known that deficiencies in Drosophila may occur spontaneously and also that they may be readily induced by x-rays. Cytological analysis of spontaneous and induced deficiencies indicates that in both types a section of the chromosome is missing. In the Notch deficiencies the

missing section varies in size from one salivary chromosome band to as many as 36 bands. Cytogenetic analysis shows that some changes which behave biologically as deficiencies have the full complement of bands, that is, they are cytologically normal. If cytological analysis is taken as a true index of the situation, the Notches which have a full complement of bands may be considered as gene mutations, and the cytologically detectable deficiencies as changes in which a section of the chromosome has been eliminated. The latter might conceivably have occurred as a result of a single event or through two independent events each causing one break in the chromosome. In this paper data will be presented showing the lengths of the Notch deficiencies and an attempt will be made to analyze the manner in which these deficiencies originated.

Material.—This analysis will include all changes which show the Notch phenotype and are not associated with chromosomal rearrangements (translocations or inversions). In addition three Notches are included which are associated with chromosomal rearrangements but are at the same time deficiencies for a segment which covers the Notch locus.

Cytogenetic studies indicate[1] that the Notch locus is represented in the salivary gland chromosomes by the band shown on Bridges'[2] map as 3C7. Thus all deficiencies which include this band are classified as Notch deficiencies. It is well known that in chromosomal rearrangements the genes in the proximity of a break are frequently affected. In our collection of 85 x-ray induced Notches there are 34 in which the Notch phenotype arose in conjunction with either a translocation or an inversion. A similar effect on adjacent loci may be expected in deficiencies where the nature of the chromosomal rearrangement is the loss of a segment. Three such cases are available in our material. In each a change in the Notch locus is associated with an adjoining deficiency which does not include the 3C7 band. These three Notches are classified as non-deficiencies. Among 48 induced Notches not associated with chromosomal aberrations, 37 are cytologically detectable deficiencies while 11 are not.

On Bridges'[2] map of the salivary *X*-chromosome a considerable number of bands are indicated as doublets, that is, they are shown as two lines very similar in appearance and placed close together. In the studies conducted in this laboratory no case has been found showing a break between two lines which are represented on the map as a doublet. Therefore in this paper all doublets are counted as single bands so that the total number of bands in the *X*-chromosome is 647.

The induced Notches originated from experiments in which males received an x-ray treatment of between 2500 and 3000 roentgens. All fertile Notches so obtained were kept and analyzed. Of the 13 spontaneous Notches, 5 were found in this laboratory while 3 were found by Doctor O. Mohr, 3 were obtained from the Pasadena Laboratory and 2

from Doctor G. Gottschewski. In table 1 the Notches are classified according to the number of bands involved in the deficiency.

TABLE 1

NOTCHES CLASSIFIED ACCORDING TO THE NUMBER OF SALIVARY CHROMOSOME BANDS INCLUDED IN THE DEFICIENCY

	NUMBER OF BANDS INCLUDED IN DEFICIENCY																			
NOTCHES	0	1	2	3	5	6	8	10	12	13	14	15	20	21	27	30	31	33	36
Induced	11	9	5	2	1	2	..	2	1	..	3	2	3	2	1	1	1	1	1
Spontaneous	2	4	2	1	2	..	1	..	..	1	..	..	..	..	..	..	..	..	..

Discussion.—It has been suggested[3] that the observed deficiencies produced by 2500–3000 roentgens which involve the Notch locus might arise by two different processes. The longer deficiencies would correspond to the usual chromosomal aberrations involving two independent breaks

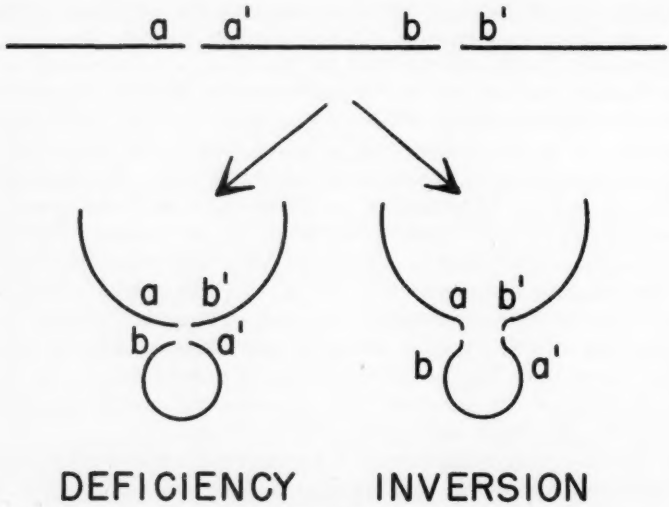

FIGURE 1

Diagram indicating symmetry in the origin of deficiencies and of inversions.

and may be classified as "two-event" processes. The short ones, which are particularly frequent, would arise from some different "single-event" process, and correspond more closely to the mutations affecting the Notch locus which are not cytologically detectable deficiencies. It is of interest to distinguish between the two groups of phenomena in order to ascertain the maximum size of the affected region involved in "single-event" processes. An attempt will be made to do so.

As a first step we shall try to calculate the expected frequency of "two-event" deficiencies involving the Notch locus. A considerable amount of data on cytologically detectable chromosomal rearrangements produced by x-rays has been published by Bauer, Demerec and Kaufmann and by Bauer.[4] Although the process involved in production of chromosomal aberrations is not yet clear, it seems reasonable to assume that any large size deficiency is just as probable as a corresponding inversion since the two phenomena (Fig. 1) arise from alternate analogous rejoining of the same chromosomal fragments. This consideration takes into account the deficiencies arising from simple two-break processes only and excludes those associated with more complicated rearrangements as well as those which are actually not deficiencies but intercalary translocations of chromosomal fragments. Actually all these types are included in the experimental material under consideration but for the sake of simplicity we shall omit multiple-break rearrangements from the following calculations. This should not greatly affect the results, since multiple-break rearrangements are much less frequent than those with two breaks only (94 and 265, respectively, in Bauer's material).

We shall calculate first the frequency of X-chromosome inversions produced by 3000 roentgens involving n bands and including any given locus. According to Bauer,[4] 104 two-break aberrations were produced by 3000 roentgens in 595 tested sperma; 412 out of 671 two-break aberrations had both breaks within the euchromatic regions of long chromosome arms; 29 out of 399 such aberrations were euchromatic X-chromosome inversions. The frequency of euchromatic X-chromosome inversions at 3000 roentgens is then approximately:

$$\frac{104}{595} \times \frac{412}{671} \times \frac{29}{399} = 0.0078.$$

According to Bauer, Demerec and Kaufmann[4] the length of inversions is distributed at random. On this basis the expected frequency of inversions involving a certain number, n, out of a total number of N euchromatic bands within the X-chromosome is:

$$\frac{2(N-1-n)}{(N-1)(N-2)} \approx \frac{2}{N-2} \quad \text{if } n \ll N.$$

The *a priori* probability that any particular band is included among those which are involved in such an inversion is approximately $n/N-2$ (the first and last euchromatic bands cannot be included). Therefore, the expected frequency of inversions or deficiencies produced by 3000 roentgens within the X-chromosome involving a small number n of bands and including any given locus is:

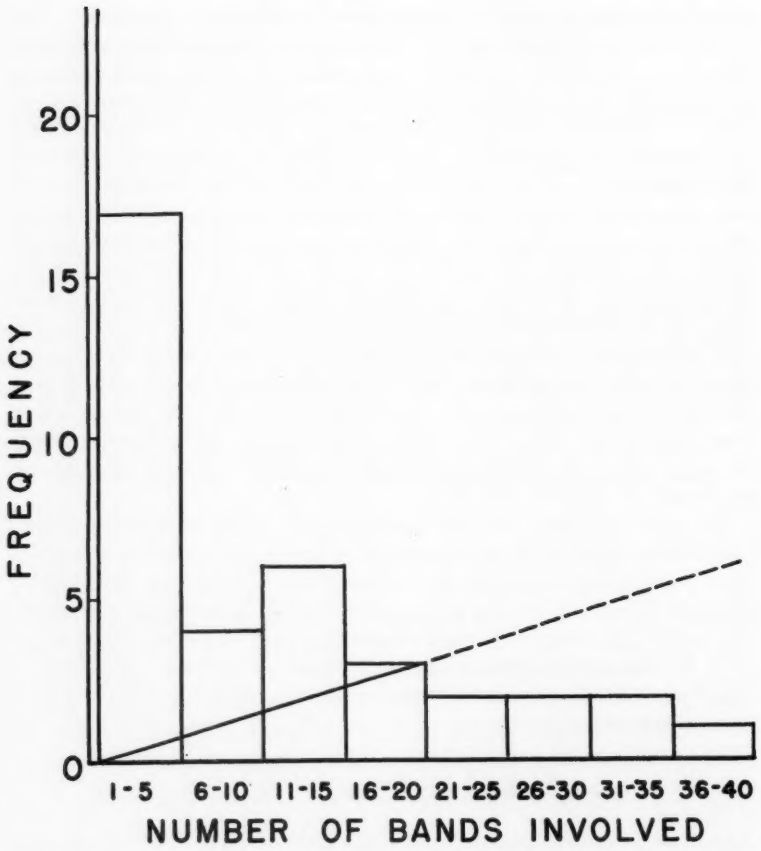

FIGURE 2

Histogram showing the frequency distribution of x-ray induced Notch deficiencies arranged according to the length of the deficient segment (data from table 1). The straight line represents the theoretical frequency distribution of deficiencies arising from two independent breaks according to formula (1). The expected frequency should be lower in the dotted region of the line on account of low viability of long deficiencies.

$$0.0078 \frac{2}{N-2} \times \frac{n}{N-2} = 0.0078 \times 2 \times \frac{n}{645^2} \approx 3.85 \times 10^{-8} \times n.$$

Material considered in this paper involves 85 fertile Notch mutations. Demerec[5] has reported finding 13 such mutations among 112,977 gametes irradiated with approximately the same dose. One can therefore assume

that about: $\frac{85}{13} \times 112{,}997 \approx 740{,}000$ gametes have been investigated in the material reported here. The expected number of deficiencies involving n bands and including the Notch locus is then:

$$3.85 \times 10^{-8} \times 740{,}000\, n \approx 0.03\, n. \qquad (1)$$

This result is expected to hold only if n is so small that no locus affecting vitality in the neighborhood of Notch is affected, that is, for $n < \approx 20$–25.

Comparison of formula (1) with the experimental results (Fig. 2) shows that the formula represents correctly the frequency of deficiencies involving more than about 15 bands. On the other hand, the number of shorter deficiencies is far in excess of the expectation, particularly in view of the fact that according to (1) the shorter deficiencies should be less frequent rather than more frequent than the longer ones. This is taken as an indication that, whereas deficiencies of 15 or more bands may be due to the same process as the large size inversions, the shorter deficiencies must be caused by a different process.

To prove that short deficiencies are due to a "single-event" process, while the larger ones correspond to a "two-event" process, it is necessary to show that the ratio of the frequencies of large to that of short deficiencies is dose-dependent. Another conceivable explanation of the high frequency of short deficiencies could be that the number of large size chromosomal aberrations is reduced by some healing process. That would mean that two breaks would have a better chance of giving rise to an aberration if they occurred within a very short distance, below a limit of approximately 15 bands. In this case the ratio of the frequency of long deficiencies to that of the short ones would not be dose-dependent.

Analysis of deficiencies among spontaneous Notches yields some evidence on this subject. The largest among 11 spontaneous Notch deficiencies involves 13 bands, whereas among 37 Notch deficiencies induced with 3000 roentgens 15 exceeded 13 bands. This suggests that large deficiencies are less frequent among spontaneous than among x-rayed Notches. A statistical test of the homogeneity of the distribution of x-ray induced and spontaneous deficiencies has been made by means of an analysis of the contingency table:

	13 BANDS OR LESS	MORE THAN 13 BANDS	TOTAL
X-ray induced	22	15	37
Spontaneous	11	0	11
Total	33	15	48

The χ^2-test (which gives $P \approx 0.011$) is not reliable in this case because of the small numbers which are involved. The exact treatment involves a consideration of the probability of all possible distributions of deficiencies in the four cells of this contingency table. The particular distribution which has been found had the *a priori* probability $\frac{33!\ 15!\ 37!\ 11!}{48!\ 22!\ 11!\ 15!\ 0!} =$ 0.0086. The level of significance is $P = 0.0102$. This evidence suggests that short and long deficiencies are not equally distributed among the x-ray induced and the spontaneous Notches.

More conclusive evidence should, however, be obtained by the analysis of Notches produced by a different dosage of x-rays, because only in this way can the results be directly compared with those obtained with high dosages. Experiments with this aim are in progress.

Therefore, one might assume that the length of 15 bands represents approximately an upper limit for a "single-event" deficiency. This length is about 6 μ in salivary gland chromosomes and should probably be about 100 times smaller in the sperm chromosomes, that is, approximately 600 Å $= 0.06\ \mu$. This finding is analogous to the phenomena involved in the "single hit chromatid breaks" described by Sax.[6]

It is possible that short deficiencies have the same origin as the 11 mutations of the Notch locus, which show no detectable cytological effect. The frequency of this group is approximately the same as that of the single-band deficiencies, both in the x-rayed and in the spontaneous material.

It is of interest that the critical length at which formula (1) yields a frequency comparable to that obtained experimentally is about the only one which would have been acceptable (15 to 20 bands), whereas the formula has been derived quite independently of any experiment involving Notch. The critical length should, in fact, not have been much shorter than the largest spontaneous deficiency (13 bands), since "two-event" spontaneous chromosomal aberrations are so rare that they need not be taken into consideration. Neither could it have been much longer than 20 to 25 bands because of the influence of viability factors.

Summary.—Extensive data on the frequency of deficiencies of various lengths involving the Notch locus are reported. Calculations show that deficiencies which include more than about 15 bands can be accounted for as being analogous to the large size chromosomal aberrations, that is, they are caused by two breaks induced by two independent events. It has been shown that it is very likely that most of the short deficiencies, including all the spontaneous ones, arise as a result of a different process, possibly a "single-event" process, having a radius of action of about 600 Å.

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ANOTHER CASE OF UNEQUAL CROSSING-OVER IN DROSOPHILA MELANOGASTER

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Females homozygous for the sex-linked dominant, Bar, occasionally give rise to wild-type reversions and to forms with more extreme eye reduction than Bar. This behavior was shown by Sturtevant[1] to result from unequal crossing-over. The Bar-reverted type was considered to be a deficiency for the Bar gene; while the extreme form, called Ultra-Bar or Double-Bar, was interpreted as a duplication for that gene. Later, Wright[2] suggested that Bar itself had something additional present which when lost by unequal crossing-over would give back a normal chromosome (Bar-reverted).

The cytological nature of Bar was cleared up independently by Bridges[3] and Muller, *et al.*,[4] who investigated the salivary gland chromosomes. They found Bar to be a tandem duplication, in normal order, for an *X*-chromosome section composed, according to Bridges' detailed analysis, of six bands. Bridges further demonstrated that Bar-reverted had the identical banding of a normal chromosome, whereas Double-Bar had a serial triplication for the region present twice in Bar and once in Bar-reverted.

The second case of a tandem duplication being responsible for a dominant "mutation" is that of the sex-linked Hairy wing, which Demerec[5] has shown is a repetition for a single heavy band near the tip of the *X*-chromosome. However, its location in a region of extremely low crossing-over prevented a study of unequal crossing-over.

This paper is a preliminary report on an autosomal tandem repeat which was detected as a suppressor of the dominant mutant, Star (*S*, 2-1.3).

An analysis of the salivary gland chromosomes of this suppressing factor, when homozygous, when closely paired with a normal chromosome and when present as an unpaired haploid strand, consistently showed the presence of a tandem duplication in direct order near the left end of the

second chromosome. The section present twice appears to include the two faint bands, 21 *D* 4—5, and the heavy, frequently capsulated doublet, 21 *E* 1—2; i.e., a section of at least four bands.

Dp(2)*S* appeared in a study of changes at the Star locus, a consideration of which is essential before discussing the properties of this repeat. *S*/+ has roughened, slightly reduced eyes; *S*/*S* is always lethal. Star-recessive (S^r) is the name tentatively given to a recessive mutant near if not at the Star locus. S^r/S^r has smaller, rougher eyes than *S*/+, and may have gaps at the tips of the wing veins. The compound, S/S^r, is much more extreme, having a narrow diamond-shaped eye and extensively interrupted venation.

Bridges has reported that the salivary gland chromosomes of Star are apparently normal. The same appears to be true for Star-recessive and also for the dominant Suppressor of Star (*Su*—*S*, 2—1.3±) found by Curry.

The allelic relation between *S* and S^r is, as yet, ambiguous. From *al S ho*/S^r females (*al* = aristaless, 2—0.0; *ho* = heldout, 2—4.0), wild-type "reversions," which are always heldout crossovers and which are cytologically normal, occur with a frequency of 0.01% (4 : 31, 106); by using females heterozygous for inversions in all of the other chromosome arms, their frequency has been stepped up to 0.046% (12:26, 370). Yet, a crossover complementary to the reversions has not been detected. The situation may be similar to a case, recently reported by Oliver,[6] of reversions, associated with crossing-over in one direction, arising from females carrying two alleles of the lozenge eye mutation. For the sake of simplicity, *S* and S^r are considered as alleles in this paper.

Dp(2)*S* arose spontaneously as a single individual among approximately 49,000 offspring of *al* S^r *ho*/S^r females individually mated to *al S ho*/*Cy*, *E*—*S* males. The fly had normal aristae, nearly wild-type (Star suppressed) eyes and heldout wings. Tests showed that the mother had contributed al^+, *ho* and *Dp*(2)*S*, whose origin was therefore associated with crossing-over. *Dp*(2)*S*/+ and *Dp*(2)*S*/S^r look wild-type. *Dp*(2)*S*/*Dp*(2)*S* is also normal except for an occasional slight extra vein near the fifth longitudinal vein. This wild-type action is in striking contrast to the pronounced phenotypic effects of Bar and Hairy wing.

A study of unequal crossing-over in the heterozygous duplication has shown that the Star locus is included in the repeated sections; i.e., it has been possible to recover from *al Dp*(2)*S ho*/*S* females unequal crossover products which have *S* inserted into the left (distal) section of the repeat, and others with *S* introduced into the right (proximal) region. The latter occur with a frequency of 0.3% (6:1948) or roughly thirty times as frequently as the former (0.01% or 2:*ca* 23,000). In terms of genetic length this indicates that the *S* locus is included in the extreme right portion of

each of the two regions present in duplicate. The original duplication, since it arose from homozygous S^r, might be expected to have a S^r gene in each of these positions. Using parentheses to bound the repeated regions, its composition may be written: $Dp(2)S = (\ldots S^r.)(\ldots S^r.)$. That S^r is present in the proximal section has been demonstrated by its recovery from

$$\frac{al\ (\ldots S^r.)(\ldots S^r.)\quad ho}{(\ldots S.)}$$

females as S^r *ho* cross-overs, whose cytological picture is normal; their frequency is 0.35% (57:16,568) or approximately that of the complementary *al* $(\ldots S^r.)(\ldots S.)$ class mentioned above. In the two cases where S was inserted into the left section, the product may be written: $(\ldots S.)$-$(\ldots S^r.)$ *ho*, and its origin visualized as the result of the following pairing:

$$\frac{al\ (\ldots S^r.)(\ldots S^r.)\quad ho}{(\ldots S.)},$$

accompanied by a cross-over between the S locus and the break point of the duplication. The complementary crossover is expected in this case to be *al* S^r, or the removal of S^r from the left section. Yet, although a total of 243 S^r *ho* types have been detected, no cases of *al* S^r have occurred. This may mean that S^r is slightly to the right of S, as was suggested, in part, by evidence given above. On this basis, either S^r is just outside the duplication or it is so close to the break point that crossing-over has failed so far to remove it from the left section.

Although the phenotypic effects of the original duplication are consistent with the assumption that one S^r and a normal allele of S^r are acting, the origin of $Dp(2)S$ from homozygous S^r would seem to indicate that this action is more likely a position effect. A preferable notation, for the present, would be $Dp(2)S = (\ldots S^r?.)(\ldots S^r.)$.

From *al* $(\ldots S^r?.)(\ldots S^r.)$ *ho*/+ females, normal S^r *ho* chromosomes and *al* $(\ldots S^r?.)(\ldots S^+.)$ occur with approximately equal frequencies as expected.

There is genetic evidence, not of a crucial character, that the locus of net (2—0.3±) is also included in $Dp(2)S$ at the extreme left end of each section. If this is the case then the total frequency of crossing-over between the loci of net and Star in heterozygous $Dp(2)S$ is greater when the distal section is involved (1.4%) than when the proximal one takes part (0.7%). As in the experiments previously given, these data are obtained from females heterozygous for inversions in some of the other chromosome arms with the result that the normal *net*–*S* distance of 1% is increased to 2% or more.

From females homozygous for the original duplication whose repeated sections may be supposed occasionally to pair in an unequal manner, diagrammatically,

$$\frac{al\ (\ldots S^{s}?.)(\ldots S^{s}.) \qquad ho}{(\ldots S^{s}?.)(\ldots S^{s}.)},$$

two types of unequal cross-over products have been obtained which, apart from phenotype, are analogous to the derivatives produced by homozygous Bar females. The normal chromosome products, corresponding to Bar-reverted, are detected on the basis that they carry S^{s}; their frequency is 0.25% (9 *al* S^{s} + 5 S^{s} *ho*: 5594). New chromosomes with three sections in tandem repetition, as is the case with Double-Bar, occur with approximately the same frequency as the normal types, namely, 0.18% (8 *al* + 3 *ho*, triplications: 6000); their action is to suppress, completely, *S E–S* (*E–S* = Enhancer of Star, 2- 6.±), whereas *Dp*(2)*S* (...S^{s}?.) (...S^{s}.) only partially suppresses the small rough eye effect seen in *S E—S*/+. The unequal crossover types have been examined in the salivary gland chromosomes and the analogy with the Bar derivatives has been found to hold. The homozygous triplication, symbol, *Tr*(2)*S*, has slightly bulging eyes with large facets; in addition to occasional slight extra veins, described for the homozygous *Dp*(2)*S*, there is often a branching of the second longitudinal vein. The wing effects are perhaps to be ascribed to the locus of net.

Homozygous *Tr*(2)*S* females have produced S^{s} chromosomes with only one section present, and also a new "dominant" unequal crossover product, which over S^{s} (or S^{s+}) has eyes resembling those of homozygous *Tr*-(2)*S*. A cytological analysis supports the conclusion that the "dominant" is a repeat of five sections. When homozygous this quintuplication, symbol, *Qn*(2)*S*, is still quite viable and fertile, and has the same effects, but much more intensified, as homozygous *Tr*(2)*S*. *Qn*(2)*S* would correspond to Quadruple-Bar obtained by Rapoport[7] from attached-*X* females homozygous for Double-Bar.

Summary.—1. An autosomal tandem duplication is described, whose origin was associated with crossing-over.

2. Genetic evidence indicates that the locus of Star and possibly the net locus are included in each of the duplicate sections.

3. The homozygous duplication gives unequal crossover products analogous to Bar-reverted and Double-Bar. A repeat of five sections has been derived from the homozygous triplication.

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*POSITION OF ADULT TESTES IN DROSOPHILA MELANOGASTER MEIGEN**

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In Drosophila the effects of irradiation on genetic constitution can be readily investigated by irradiating mature sperm while it is still within the body of the male and then analyzing whatever chromosomal or genic alterations become apparent in the progeny of the treated flies. In order to reach the sperm, the rays must penetrate the intervening tissues of the fly. When rays of weak penetrating power (soft x-rays, ultraviolet rays, protons, alpha particles, cathode rays) are to be used, it becomes particularly desirable to know the position of the sperm-containing organs relative to the surface of the body and thereby to determine how maximal exposure to effective radiation may be attained. To meet this need, a study has been made of the natural position of the testes and of the effect of external pressure on their arrangement, in wild type (Swedish-*b*) adult males of *D. melanogaster*. The technique employed included dissection of living and fixed material, direct observation on intact specimens and study of serial sections. Fricke and Demerec[1] have found that flies that have had their abdomens flattened to as little as 0.15 mm. during irradiation may recover and function normally. To study the effect of such treatment on the position of the testes, the abdomens of etherized flies were pressed between a slide and cover glass, which were separated by another cover glass, 0.12 mm. in thickness, to limit the degree of flattening. These flies were studied alive or were fixed while compressed and then dissected. Details of the testes are rendered discernible through the body wall of flattened flies by the addition of glycerin, but this soon kills the specimen.

Morphology and Normal Position.—The internal reproductive system of the male (Fig. 1) consists of paired testes, seminal vesicles, vasa deferentia (vasa efferentia of other authors) and accessory glands, and an unpaired ejaculatory duct with an appended ejaculatory bulb ("sperm pump"). The ejaculatory duct consists of two portions: a dilated, thick-walled anterior portion (vas deferens of other authors) extending from the vasa

deferentia to the ejaculatory bulb and a slender, thin-walled posterior portion (ejaculatory duct of other authors) extending from the bulb to the aedeagus. The revised terminology is introduced to conform with that of general insect morphology (see Snodgrass,[2] Weber[3]). If the term "vas efferens" is used, it must be limited to the short narrow duct connecting testis and seminal vesicle (*x*, Fig 1, here designated as "the testicular duct") since this is the only part that can be regarded as corresponding to the vasa efferentia of compound testes, where such a duct is present at the base of each sperm tube. The two "testicular ducts" in Drosophila can also be regarded as remnants of the mesodermal vasa deferentia of more generalized insects, for they are said to be of mesodermal origin while the remainder of the duct system (including seminal vesicles) is ectodermal.[4, 5] In the adult, the inner epithelium of the testicular ducts is markedly thicker than that of the seminal vesicles and vasa deferentia. The term "vasa deferentia" is here applied in a functional sense to the paired ducts extending from the testicular ducts to the median ejaculatory duct, since they correspond in position to the paired vasa deferentia of other insects, regardless of their developmental history. The seminal vesicles are simple dilatations of the vasa deferentia.

All the organs are colorless or nearly so in recently enclosed individuals, but testes, seminal vesicles and vasa deferentia gradually become a bright butter-yellow. The color fades in fixed material. Spermatozoa are found in the testes, seminal vesicles and vasa deferentia; they apparently are not stored in any of the other organs.

Each of the two testes is a single long sperm tube, the basal half helically coiled and the distal half forming a freely extended arc. The coiled por-

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EXPLANATION OF FIGURES

Drawings based upon camera lucida tracings of dissections and whole mounts; magnification × 33.

Fig. 1. Male reproductive system of *Drosophila melanogaster*, dorsal aspect; from an old individual with well-distended seminal vesicles. Organs spread apart but arranged to show their relative positions; successive gyres of each testicular coil separated and testicular duct drawn out of its normally central position within the first gyre.

Fig. 2. Dorsal dissection of male with distended crop, showing reproductive system in place and typical transverse position of testes. Overlying parts of alimentary canal indicated by dotted lines.

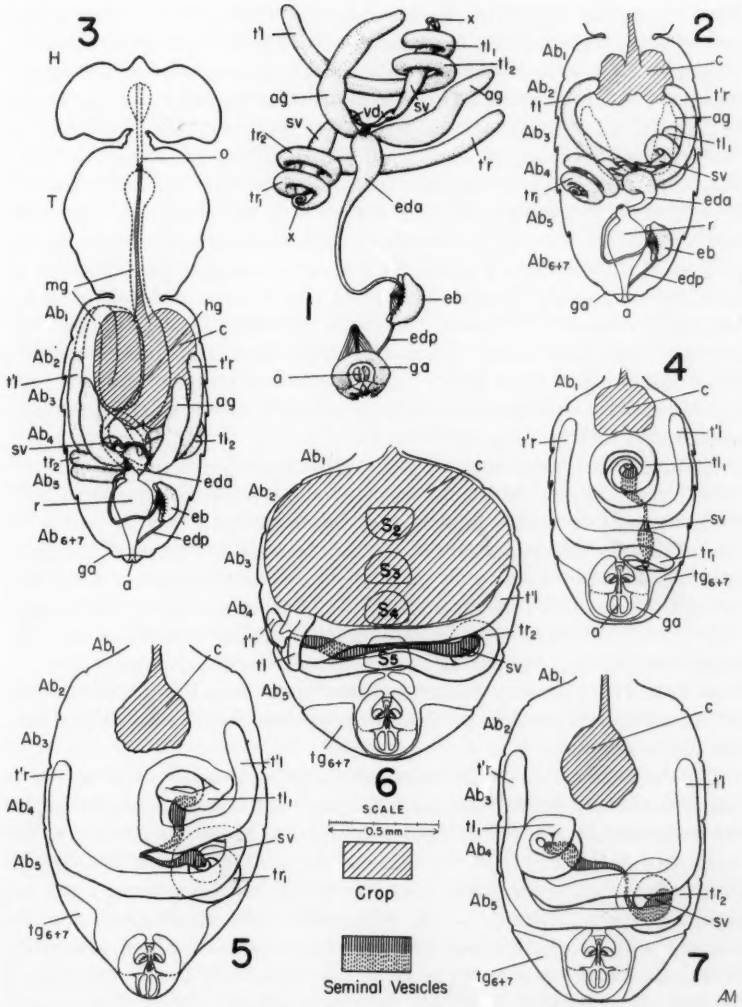
Fig. 3. Dorsal dissection of abdomen of male with undistended crop, showing oblique position sometimes assumed by uncrowded testes, especially in young individuals.

Fig. 4. Ventral aspect of intact abdomen of desiccated male (deprived of food and water for 48 hours), showing rearrangement of testes due to contraction of venter of anterior segments.

Fig. 5. Ventral aspect of artificially depressed abdomen of a recently eclosed teneral male having both testicular coils on left side.

Fig. 6. Ventral aspect of artificially depressed abdomen of male with distended crop.

Fig. 7. Ventral aspect of artificially depressed abdomen of desiccated male.



a, anus; *ag*, accessory gland; Ab_{1-7}, first to seventh abdominal segments; *c*, crop; *eb*, ejaculatory bulb; *eda*, anterior ejaculatory duct; *edp*, posterior ejaculatory duct; *ga*, genital arch; *H*, head; *hg*, hind-gut; *mg*, mid-gut; *o*, oesophagus; *r*, rectum; s_{2-5}, second to fifth abdominal sternites; *sv*, seminal vesicle (dilatation of vas deferens); *T*, thorax; tg_{6+7}, fused sixth and seventh abdominal tergites; tl_1, tl_2, first and second gyres of left testicular coil; *t'l*, tip of left testis; tr_1, tr_2, first and second gyres of right testicular coil; *t'r*, tip of right testis; *vd*, undistended portion of vas deferens; *x*, testicular duct.

tion is spirally wound twice about a center occupied by the testicular duct and by some portion of the seminal vesicle. The testicular duct describes a minute, more or less complete third gyre. In natural position, the undistended vesicle of young flies lies more or less obliquely within the second gyre of the coil (gyre nearest the free end of the testis). When distended, the vesicle may extend farther out of the coil. Extreme distention of the vesicle, to as much as two or three times the diameter of the testis, is sometimes found; this pushes the coils toward their attached end. The seminal vesicle tapers into the twisted vas deferens which enters the dilated portion of the ejaculatory duct. It is to be noted that the testes do not form flat spirals: the two principal gyres of a coil lie side by side and not one within the other as some previously published figures intimate.[5, 6, 7] In freshly emerged, teneral flies the testes are turgid, thick and have proportionately less of their length extending freely from the coil; the seminal vesicles are small and undistended; and the accessory glands are also smaller than they are later.

Each testis contains spermatogenic cells and mature sperm. The extreme tip is occupied by a small mass (about 50 μ long) of spermatogonia. Spermatocytes are most conspicuous in the distal free half of the testis and extend into the adjoining gyre of the coil, but are not found in the basal gyre. Bundles of developing and fully formed spermatozoa are found throughout the testis, except apically in the short spermatogonial region. Only those in the coil show definite, deeply staining mature heads. The sperm bundles are longitudinally arranged and less crowded in the free portion of the testis, densely massed and much convoluted in the coil. They become still more densely packed in the seminal vesicles, causing distention of these organs.

The normal position of the testes within the abdomen is asymmetrically bilateral, each with the coiled portion lying ventrolaterally on one side of the body and the free end extending to the opposite side. The testes are designated as left and right according to the position of their tips, for Dr. Curt Stern informs the writer that the tip lies on the side originally occupied by the entire testis in the early pupa. The tip thus indicates the primary position of the testis, while the coiled portion, added posteriorly to the ellipsoidal pupal testis, acquires its position on the opposite side of the body secondarily. The vasa deferentia are also reversed in position during development and each becomes twisted by the spiral growth of the testis to which it is attached.[8] The axes of the testicular coils parallel, more or less, the longitudinal axis of the body, so that the loops lie in a transverse vertical plane. The left testis lies more anterior than the right, the free part of the left crossing the body in front of that of the right. The point of union between testis and seminal vesicle is directed cephalad in the anterior testis, caudad in the posterior testis. Viewed from behind,

the posterior testis is coiled in a counterclockwise direction about its axis with the second, more distal gyre anterior to the first, while the anterior testis is coiled clockwise and the spiral progresses caudad. It is apparent, however, that when viewed from the base the direction of coiling from base to tip is the same in both testes, viz., counterclockwise and toward the ejaculatory duct; the testes are not mirror images of each other. *In situ* the anterior coil is dextrorotatory and the posterior levorotatory because in the former the seminal vesicle is directed caudad, in the latter cephalad (see Fig. 1). The free ends of the testes cross the ventral side of the body and then extend upward and forward laterally. The left and right accessory glands lie mediodorsal to the free end of the corresponding testis on either side. Asymmetry is further evident in the ejaculatory duct which loops over the rectum from left to right, due to a complete revolution of the terminal abdominal segments during pupal development.[5] The relative positions of the parts are indicated in figure 1, where they are shown spread apart for clarity; ordinarily the gyres of each coil are closer together than shown, the testicular duct lies within the first gyre, and the twists of the vasa deferentia are more loosely disposed.

The segmental position of the testes is subject to individual variation, apparently conditioned mainly by the expansion of the crop that lies in the anterior part of the abdomen. The volume of the crop is dependent upon the age and nourishment of the fly. In flies that have just emerged and have not fed, the crop is unexpanded and lies in the ventral part of the first and second abdominal segments. After feeding, the crop becomes greatly distended with liquid contents, appears grayish and translucent through the body wall, and may extend back as far as the caudal part of segment 4. The abdominal viscera are then crowded above and behind the crop, and the entire abdomen appears swollen. If distended flies are deprived of food and water for 24 to 48 hours, the crop decreases in size, the abdomen contracts and becomes ventrally concave, and the viscera are more loosely disposed.

The coiled portions of the testes may, accordingly, be incompact and lie in abdominal segments 3 to 5 (Fig. 2), or be compressed and crowded back into 4 and 5, 5 alone or even partly into 6 (Fig. 3). In specimens with small crops the coil of the left testis may lie in segment 3, or 3 and 4, the right in 4 or 5. In distended flies the left usually lies in the posterior part of segment 4, the right in 5; in extreme cases both coils may be pressed back into segments 5 and 6. When the crop is small, particularly in teneral flies, the testicular coils may deviate from their transverse position so that the plane of the coil may lie oblique to the longitudinal axis and lateral body wall (cf. Fig. 2). In flies deprived of water for two days, when the abdomen becomes ventrally concave except at its base and apex, the anterior (left) testicular coil may lie in a horizontal plane on the median line

while the right coil retains a more vertical position in the less depressed terminal part of the abdomen (segments 5 and 6) (Fig. 4). Normally, however, only the outer and ventral portions of the coils lie against the body wall. The free ends of the testes may reach the anterior border of segment 2 or the tips may lie as far back as segment 3, expansion of the crop compressing them against the lateral walls of the abdomen. Sections show that external to the testes lie a layer of adipose tissue (14–50 μ thick, dehydrated), ventral and lateral muscle fibers (3–4 μ), the very thin remnant of the epidermal layer (0.7 μ), and the cuticula (1.5–4 μ).

Aside from the influence of the crop, flies occasionally show abnormally asymmetric disposition of the testicular coils. Both may lie on the left side of the abdomen or both on the right (cf. Fig. 5). Gleichauf[5] recorded 6.67% of the former and 4.44% of the latter in 135 wild type flies examined, 0–11.98% and 1.69–5.55%, respectively, in 340 flies of three mutant strains ("white," "black" and "quintuple"). He also mentions a case in which the craniocaudal relations of the left and right testes were reversed.

Effect of External Pressure.—When the abdomen is artificially depressed or compressed, the testes tend to flatten in place. However, considerable displacement may occur when the crop is small and pressure is unevenly applied. In distended flies dorsoventral pressure extends the testes transversely in segments 4 and 5, or in segment 5 alone, with the coils pressed down irregularly and rarely having room to form flat spirals (Fig. 6). In desiccated flies, with contracted crops and flaccid abdominal contents, even pressure flattens the coils in a horizontal plane somewhere in segments 3 to 5; more or less overlapping of the gyres persists in each testicular coil (Fig. 7). If pressure is applied progressively caudocephalad, both testes shift forward so that, e.g., the anterior coil comes to lie in segment 2, the posterior coil flattens out in segments 3 to 5. It is even possible to displace both testes to the undepressed base of the abdomen. In more turgid teneral flies displacement is less marked, and the testes again occupy segments 3 to 5 when depressed (Fig. 5). In all three types of flies the free distal portions of the testes are ordinarily well extended and applied directly against the body wall for most or all of their lengths.

Lateral pressure varies similarly in its effects according to the condition of the flies. The testicular coils flatten down somewhere in the ventral halves of segments 3 to 6, the left and right sometimes overlapping. The degree of spreading of the coils depends upon the space available within the abdomen.

Since reduced turgidity of the abdomen facilitates the flattening and maximal spreading of the testes under pressure, and since the testes lie closest to the ventral and lateral body walls, it is concluded that the optimal exposure of the entire testis to poorly penetrating radiation should be attained by irradiating the ventral surface of undistended flies when the

abdomen has been evenly flattened by dorsoventral pressure. Under these conditions, the only tissues intervening between the source of irradiation and the greater part of the testes are the epidermal cuticula, a thin discontinuous layer of muscle fibers of the body wall, and probably a small amount of compressed adipose tissue. The sperm are further shielded by the very thin (1.5 to 2 μ) two-layered epithelial sheath of the testis and, in some places, by intervening spermatogenic cells (6–22 μ thick). The greatest amount of overlapping of parts of the testes occurs in the coils; the free distal portions, where probably most imaginal spermatogenesis takes place, are well exposed by pressure even in distended flies. The parts wherein mature spermatozoa accumulate, viz., the bases of the testes and the seminal vesicles, are almost always partly covered by other portions of the testes.

Summary.—The coiled testes of *D. melanogaster* are usually bilaterally but asymmetrically located in the second to fifth abdominal segments; their exact extent and position are not fixed, but may vary according to the fly's age and nutritive condition (especially as reflected in the size of the crop). Only certain portions of the testes are normally close to the surface of the body; artificial depression of the abdomen tends to arrange them more favorably for ventral irradiation with poorly penetrating rays.

* The author wishes to express appreciation to Dr. M. Demerec for suggesting and facilitating this investigation, and to Drs. T. Dobzhansky, C. Stern and R. E. Snodgrass for helpful criticism of the manuscript.

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AN ANALYSIS OF X-RAY INDUCED SINGLE BREAKS IN NEUROBLAST CHROMOSOMES OF THE GRASSHOPPER (CHORTOPHAGA VIRIDIFASCIATA)

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Of considerable importance in furnishing information regarding the mode of action of x-rays in inducing structural changes in the chromosomes is the quantitative analysis of single breaks. Sax,[1] who has made extensive studies of single as well as multiple breaks in chromosomes of the first microspore division in Tradescantia, has concluded, from the relation of dosage to breakage, that the chromosomal break is the result of a single event or "hit." Most animal cells, however, do not lend themselves readily to analyses of single breaks. Drosophila, despite very extensive studies of x-ray induced salivary gland chromosome aberrations, has yielded extremely few positive cases of terminal deletions, possibly because these are somehow eliminated during the many mitoses intervening between their formation and the laying down of the rudiments of the salivary glands. In the grasshopper neuroblast, however, it is possible to identify breaks in the same mitotic cycle in which they are produced, and so there is less chance for their disappearance between treatment and observation.

A previous study[2] of the effects of x-rays on the neuroblast chromosomes of *Chortophaga viridifasciata* showed that translocations are not infrequently produced by treatments of 250 r and above, while they are rare after 125 r, a treatment that does, however, give on the average one to two single breaks per cell. In the present study, therefore, the maximum dose given was 125 r, and this was successively halved to give doses of $^1/_2$, $^1/_4$, $^1/_8$ and $^1/_{16}$ this amount.

At the time of treatment the eggs contained embryos of an age equivalent to two weeks at 26°C. After irradiation they were kept moist at 26°C. When sufficient time had elapsed that neuroblasts in interphase or early prophase at irradiation had reached anaphase and early telophase, embryos were dissected out of the eggs in physiological salt solution at hour or half-hour intervals, fixed and stained in acetocarmine and mounted in an acetocarmine-karo mixture. It will be noted in table 1 that the interval of time elapsing between irradiation and fixation was increased as the dosage became greater. This is necessitated by the greater delay in mitosis occasioned by increased doses of x-rays. It will also be seen that the time intervals between irradiation and fixation range from 1–3 hours after the different doses. Series that included greater ranges after certain of these doses showed that the first cells to reach anaphase and telophase after the

end of the mitotic cessation resulting from treatment exhibit very few breaks and these are mostly chromatid effects. In a short time, however, the percentage of breakage rises to a level which does not change appreciably over the range indicated and only data within this range were included in the present study.

Irradiation was produced by a Coolidge Universal X-ray Tube operating at about 80 kv. p. and 5 ma. The rays were filtered by $^1/_8$ mm. of aluminum. The material to be treated was placed about 19 cm. from the middle of the target. Under these conditions the output was approximately 200 r/min. All doses were measured by a Victoreen dosimeter simultaneously with treatment.

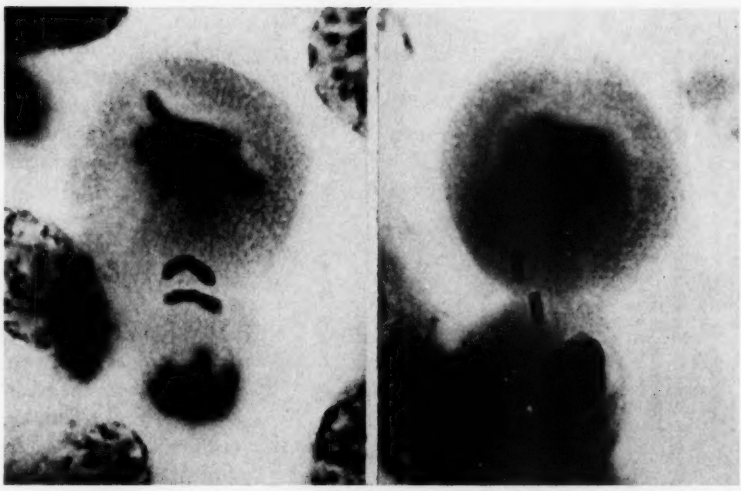

FIGURE 1

Photographs of neuroblast cells in late anaphase, each with a pair of sister chromatid fragments lacking spindle attachments. Dosage 125/2 r. × 1320.

The behavior of x-ray induced fragments lacking spindle attachments in grasshopper neuroblasts has been described previously.[2,3] Sister fragments of this kind are shown at early telophase in figure 1. Equal fragments such as these are assumed to result from one "hit" by the breaking either of a single chromosome strand before doubling has occurred or both strands of a double chromosome at the same place; each of these cells, therefore, was accredited with one break. When chromatid effects are identifiable occasionally as complete breaks, they have been included in the counts. Alterations in the anaphase chromosomes ranging in appearance from slight constrictions to long thread-like connecting strands be-

TABLE 1

SUMMARY OF EXPERIMENTAL DATA

Expected numbers are enclosed in parentheses. Poisson distributions and expected total numbers of breaks are calculated from observed total numbers for each dose.

DOSAGE IN r		125/16	125/8	125/4	125/2	125
Egg pods		2	1	2	1	1
Hours after irradiation		5–7	5–7	6.5–9	8–11	10–11
Embryos		19	8	9	12	5
Cells		696	241	185	320	89
Chromosomes		16,406	5613	4322	7421	2055
Cells with 0–5 breaks per cell	0	639 (639.3)	210 (208.5)	135 (138.2)	174 (178.5)	27 (25.9)
	1	55 (54.3)	27 (30.2)	47 (40.3)	112 (104.2)	29 (32.0)
	2	2 (2.3)	4 (2.2)	2 (5.9)	28 (30.4)	23 (19.8)
	3	0 (0.07)	0 (0.1)	1 (0.6)	5 (5.9)	6 (8.1)
	4	0	0	0 (0.04)	1 (0.9)	3 (2.5)
	5	0	0	0	0 (0.1)	1 (0.6)
Total breaks and standard errors		59 ± 7.7 (53.2)	35 ± 5.9 (36.4)	54 ± 7.3 (56.1)	187 ± 13.6 (192.6)	110 ± 10.5 (106.7)
Per cent breaks and standard errors		0.36 ± 0.05	0.62 ± 0.11	1.25 ± 0.17	2.52 ± 0.18	5.35 ± 0.51

tween adjacent parts of the chromosomes suggest incomplete breakage or reattachment, but because of the difficulty of interpreting these correctly and of distinguishing them from the secondary constrictions normally occurring on certain chromosomes in this species, no attempt was made to count or classify them. Spontaneous breakage is extremely rare in these cells; for in hundreds of anaphases and telophases examined in untreated material I have seen no evidence of it.

A summary of the data obtained is shown in table 1. On the basis of the observed number of breaks after each dose, the Poisson distribution was applied as a test to the frequencies of breaks per cell. The expected values obtained appear in parentheses beside the observed values. The close agreement for each of the several doses is strong evidence that chromosomal breaks are produced by single events acting entirely at random.

A test has been made to determine whether the differences in chromosomal breakage in different embryos is to be attributed to errors in random sampling or to biological variability. Using the data given in table 2, X^2 was calculated for each of the doses. Since all the values of P are above 0.3, the conclusion seems justified that, despite the fact that the different doses were given to embryos of different egg pods, no appreciable variability occurs other than that due to random sampling.

TABLE 2

DISTRIBUTION OF BREAKS BY EMBRYOS

	125/16 r		125/8 r		125/4 r		125/2 r		125 r	
EMBRYO	CHROMOSOMES	BREAKS	CHROMOSOMES	BREAKS	CHROMOSOMES	BREAKS	CHROMOSOMES	BREAKS	CHROMOSOMES	BREAKS
1	1081	4	644	4	46	3	575	14	759	34
2	828	2	960	6	384	1	768	24	529	32
3	207	0	1035	6	253	4	696	13	192	13
4	759	3	621	5	621	7	529	15	345	20
5	851	3	575	2	690	9	92	2	230	11
6	1008	3	368	3	912	11	989	27		
7	720	3	690	4	575	8	805	21		
8	408	2	720	5	529	10	897	28		
9	624	2			312	1	529	7		
10	1080	4					713	11		
11	1224	6					299	6		
12	1080	7					529	19		
13	1056	3								
14	864	1								
15	920	6								
16	504	1								
17	1518	4								
18	984	3								
19	690	2								
Total	16406	59	5613	35	4322	54	7421	187	2055	110
X^2	9.319		1.354		8.639		12.515		2.541	
P	0.95–0.98		0.98–0.99		0.3–0.5		0.3–0.5		0.5–0.7	

Figure 2 represents graphically the relation between dosage and per cent of chromosome breakage. There appears to be no threshold below which breaks do not occur. The relation is linear; the number of breaks is directly proportional to the dosage expressed in r-units and, therefore, to the number of ionizations per unit volume. On the assumption that the frequency of breakage per unit of radiation is independent of the dose, the number of breaks per dosage unit for the experiment as a whole is $\frac{\Sigma x}{\Sigma ND}$, in which x is the observed number of breaks, D the dosage, and N the number of chromosomes examined. The expected numbers of breaks for each treatment (table 1, Fig. 2) were calculated from this value.

On the basis of these results it is possible to secure an estimate of the re-

lation between the number of ion pairs produced and breakage. It should be emphasized, however, that such calculations represent only approximations because of certain unknown factors involved. A certain proportion of breaks may reheal between treatment and fixation and so fail to be detected at all. Sax[4] has pointed out that this is the most likely explanation of the differential effects of x-raying at different temperatures and different intensities in the production of exchange breaks in Tradescantia. Also, it is quite possible that breakage is produced by excitation alone or in combination with ionization. In either case the number of ionizations would

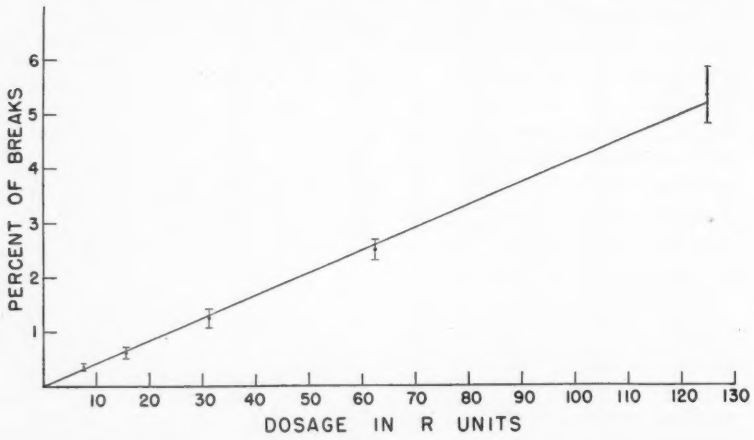

FIGURE 2

The relation between x-ray dosage and the production of single chromosomal breaks.

be no direct measure of the effective events that lead to breakage. Further, at the time of treatment the interphase or early prophase chromosomes are in so extended a state that it is not possible to compute their volumes. The number of ionizations per break may, however, be calculated for the metaphase chromosome, the volume of which may be of the same order of magnitude as that of the chromosome at treatment.

According to the dosage-breakage relation shown in figure 2 a dose of 2408 r would be required to produce 100% breakage or an average of one break per chromosome. In terms of ion pairs (1 r = 2 ion pairs/μ^3) this means that, in a medium-sized metaphase chromosome 1.5 μ in diameter and 6 μ in length and therefore with a volume of approximately 10.6 μ^3, a mean dose of 51,050 ion pairs would be required on the average to produce one surviving break. It seems very improbable that the volume of the metaphase chromosome is of the order of 51,050 times the volume of the

chromosome at treatment. Unless, therefore, there is an enormous amount of rehealing or failure of potential breaks to become actual breaks, it can only be concluded that the number of ionizations produced within the chromosome is greatly in excess of those effective in producing breaks.

Summary.—The hypothesis that the single chromosomal break is the result of a single event, namely, a single ionization or excitation, which occurs entirely at random, is supported by (1) the linear proportionality existing between dosage measured in r-units and number of chromosomal breaks, (2) the apparent absence of any threshold below which breaks do not occur, (3) the close correspondence between the observed and expected distribution of the breaks among different cells after each of the doses. The analysis of single breaks in terms of ion pairs per chromosome indicates that only a small proportion of the ionizations produced by x-rays is effective in the production of breaks.

I am greatly indebted to Dr. Ugo Fano for aid in the statistical treatment of the data contained in this paper.

* Rockefeller Fellow in the Natural Sciences.

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DISCOVERY OF A PREDICTED GENE ARRANGEMENT IN DROSOPHILA AZTECA

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A method which under certain conditions permits prediction of as yet undiscovered gene arrangements was described a few years ago.[1,2] Its essence is as follows. If an inversion transforms the gene arrangement *ABCDEFGH* into *AEDCBFGH*, a second inversion taking place in the altered chromosome may be of the overlapping type, that is one of the breaks of the second inversion may lie within and the second break outside the limits of the first inversion. Thus the arrangement *AEDCBFGH* becomes *AEDGFBCH*. If the first and the third of these arrangements are known, one can postulate the existence of *AEDCBFGH* as a necessary intermediate step. If a family of gene arrangements related as overlapping inversions is known, a phylogenetic scheme indicating the lines of descent within this family may be drawn. Occasionally such phylogenetic schemes

involve hypothetical links constructed with the aid of the method just outlined. The purpose of the present note is to record an instance of the discovery of such an hypothetical link, and certain related facts.

Six gene arrangements were found in the *A*-chromosome, an autosome, of *Drosophila azteca*.[3] They are designated as Alpha, Beta, Gamma, Delta, Epsilon and Eta. Their relationships are as indicated in the scheme in the upper right corner of figure 1. Any two arrangements which are adjacent in this scheme produce, when present together in the salivary gland cells of a larva, a characteristic single inversion loop; any two arrangements removed in the scheme by two steps produce a double inversion loop, etc. The arrangements Epsilon and Eta were found to give a double loop, an analysis of which permitted devising an hypothetical arrangement, Zeta, which had not been detected in the material then available.

Drosophila azteca was known to occur from central Mexico (Durango) to Guatemala, and in two localities north of the San Francisco Bay, California.[3] Recently it has been found at San Miguelito, Nuevo Leon, Mexico (Mr. Bob Camp), at Huachuca Mountains, Arizona (Professor Carl Epling), at Stony Creek, Sequoia National Park, California (Mr. W. Hovanitz) and at Deer Creek, south of the Lassen National Park, California (Professor Carl Epling, Mr. E. Berkoff and the writer). The distribution of this species in Mexico and Guatemala is probably continuous along the mountain ranges, but in Arizona and California the distribution is very spotty, since despite extensive collecting the species has not been found except in the few places indicated above. An examination of the salivary gland chromosomes showed that Zeta, the once hypothetical gene arrangement in the *A*-chromosome, is present in three strains from Stony Creek, two strains from Huachuca Mountains and one strain from Nuevo Leon (Fig. 1). Eta arrangement is found in two strains from Deer Creek (which are homozygous for this arrangement), in two strains from Stony Creek and in three strains from Huachuca. Epsilon arrangement is found in all three strains available from Nuevo Leon, and Alpha arrangement in one strain from the same locality.

Figure 1 shows that only phylogenetically closely related arrangements in the *A*-chromosome tend to occur in any one geographical region. Thus, Beta arrangement is confined to central and southern Mexico and to Guatemala. Alpha occurs with Beta, but extends northward as far as Durango and Nuevo Leon. Gamma and Delta are so far known only from Durango, in north-central Mexico. Epsilon and the formerly hypothetical but now actually encountered Zeta extend from Nuevo Leon to California, while the extreme member, Eta, is found only in Arizona and California. Geographical relationships as simple as these are by no means common in other species of Drosophila in which phylogenies of gene ar-

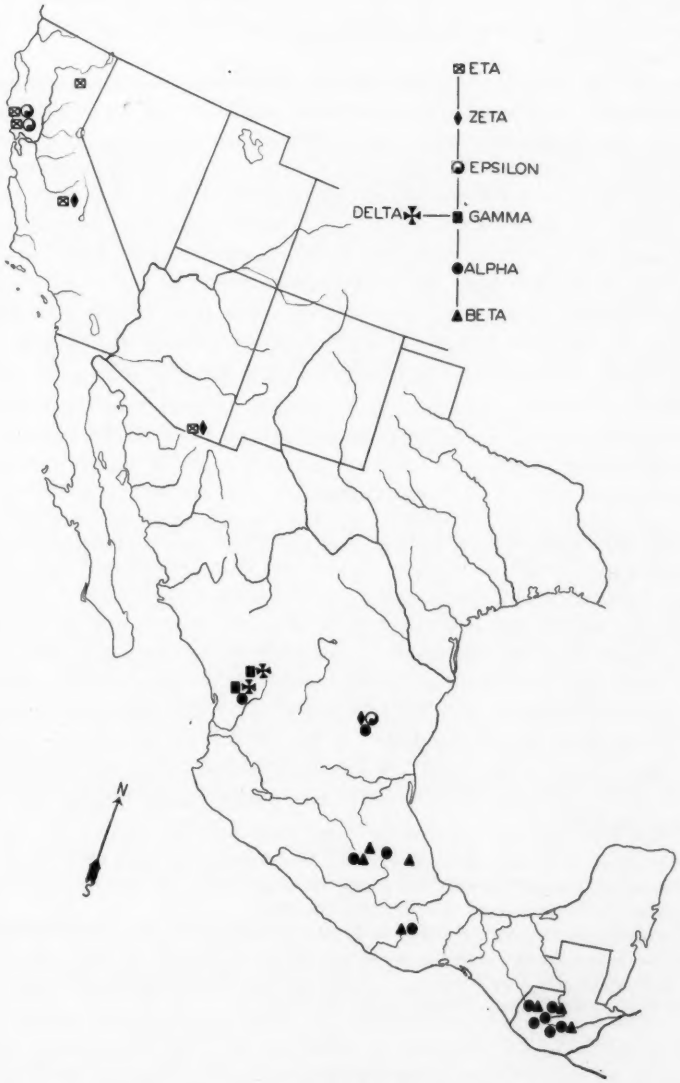

FIGURE 1

Geographical distribution of the gene arrangements in the *A*-chromosome of *Drosophila azteca*. The phylogenetic relationships of the known gene arrangements are represented in the upper right corner of the figure.

rangements have been studied; for example, in *Drosophila pseudoöbscura* phylogenetically remote arrangements frequently occur together in the same populations.

In the long limb of the *X*-chromosome of *Drosophila azteca*, two gene arrangements, Alpha and Beta, have been recorded.[3] All the strains from Mexico and Guatemala were Alpha, those from California were Beta, while in Durango both Alpha and Beta were found. All the new strains from California and Arizona are also Beta, while those from Nuevo Leon are Alpha. In the proximal part of the *B*-chromosome two arrangements, Alpha and Beta, were known, both of which were recorded from Mexico and Guatemala, and only Beta from California. The new strains from California and Arizona are pure Beta, and those from Nuevo Leon pure Alpha. In the distal part of the *B*-chromosome a family of four gene arrangements, Alpha, Beta, Gamma and Delta, was recorded. The phylogeny is:

Alpha- Beta -Delta
|
Gamma

The old data[3] showed Gamma to be the commonest in Guatemala, although occurring also in central Mexico; Alpha and Beta are common from Durango, Mexico, to Guatemala, inclusive; Delta was recorded only from Durango (one strain) and from California. The new strains from California and Arizona are homozygous for Delta, while in Nuevo Leon Alpha and Beta are found. All the available data on the geographical distribution of the gene arrangements in *Drosophila azteca* seem to make a simple and consistent story: a gradual spread from North to South, or vice versa, has taken place in the history of the species; the gene arrangements in the chromosomes have differentiated hand in hand with the distribution of the species itself.

[1] Sturtevant, A. H., and Dobzhansky, Th., "Inversions in the Third Chromosome of Wild Races of *Drosophila pseudoöbscura*, and Their Use in the Study of the History of the Species," *Proc. Nat. Acad. Sci.*, **22**, 448–450 (1936).

[2] Dobzhansky, Th., and Sturtevant, A. H., "Inversions in the Chromosomes of *Drosophila pseudoöbscura*," *Genetics*, **23**, 28–64 (1938).

[3] Dobzhansky, Th., and Socolov, D., "Structure and Variation of the Chromosomes in *Drosophila azteca*," *Jour. Heredity*, **30**, 3–19 (1939).

CRITICAL POINTS AND UNSTABLE MINIMAL SURFACES

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Minimal surfaces for which the area within the contour is stationary but not a relative minimum were noticed long ago. But it was only recently, on the basis of Morse's theory of critical points, that unstable solutions of Plateau's problem have been classified according to type numbers, the Morse relations established and, in particular, the existence of an unstable solution proved in case several relative minima exist. These results were obtained independently by M. Shiffman,[1] and by Morse and Tompkins[2] for simply-connected surfaces whose boundary Γ is subjected to certain conditions, and extension of the results for several contours are announced for forthcoming papers. A considerable difficulty to overcome consisted in the proof of a deformation property (first recognized in its significance by Morse and called by him "weak upper reducibility") of the Dirichlet functional in a function space of harmonic vectors. To attain the goal a rather deep explicit analysis of the functional is necessary.

In the present note the problem is attacked from an entirely different angle for polygonal boundaries[3] Γ; corresponding to the author's previous publications[4] a wider function space is used as a medium and explicit calculations are avoided. For the sake of brevity we confine ourselves to simply-connected surfaces. Our main result is the equivalence of the variational problem to that of finding the stationary values of a differentiable function of a finite number N of variables, where $N + 3$ is the number of vertices of the polygon Γ. An incidental result is that the type number of a simply-connected minimal surface spanning a polygon with $N + 3$ vertices cannot exceed $N - 1$.

1. *Definitions and Preliminaries.*—We consider the Dirichlet functional

$$D(\mathfrak{x}) = {}^1/_2 \int\int_B (\mathfrak{x}_u^2 + \mathfrak{x}_v^2) du dv$$

extended over the upper half-u, v-plane B with the boundary $C: v = 0$. The space $\mathfrak{S}^*$ of admissible vectors $\mathfrak{x}(u, v)$ consists of vectors continuous in $B + C$, mapping C continuously and monotonically on the polygon Γ in the $\mathfrak{x}$-space, having piecewise first derivatives in B and a finite $D(\mathfrak{x})$. The subspace of harmonic vectors in $\mathfrak{S}^*$ is called $\mathfrak{P}^*$. We may suppose that three consecutive vertices of Γ are images of three fixed points, e.g., $u = \infty$, $u = -1$, $u = 0$ on C. This normalization fixes corresponding vector spaces which we denote by $\mathfrak{S}$ and $\mathfrak{P}$, respectively. It then follows that the subspace defined by $D(\mathfrak{x}) \leq c$ is compact.[5] The other vertices of Γ correspond consecutively to values $u_1, \ldots, u_N$ on C, forming the set

(U). For a fixed set (U) we solve,[6] by a harmonic vector $\mathfrak{z}$ of $\mathfrak{P}$, the problem $D(\mathfrak{x}) =$ minimum with respect to $\mathfrak{S}$. The value of the minimum is a function

$$d(U) = d(u_1, \ldots, u_N)$$

of the N parameters u_i in the domain $0 < u_1 < u_2 < \ldots < u_n < \infty$, and $d(U)$ becomes infinite at the boundary of this domain.[7] The vectors $\mathfrak{z}$, depending on (U), which solve these minimum problems are called *minimal vectors;* they form a set $\mathfrak{M}$.

We shall consider families of variations in $\mathfrak{S}$ or $\mathfrak{S}^*$ of the following type: By a transformation, involving a small parameter ϵ,

$$\begin{aligned} u &= u' - \epsilon\Lambda \\ v &= v' - \epsilon\mathrm{M} \end{aligned} \tag{1}$$

where Λ and M are functions of u, v and ϵ or of u', v' and ϵ, the domain $B + C$ may be transformed into itself in a one-to-one way. Λ and M are continuous functions in $B + C$ and have piecewise-continuous first derivatives absolutely bounded by a bound a. The quantities Λ and M for $\epsilon = 0$ are called λ and μ. Then the vector $\mathfrak{x}$ of $\mathfrak{S}^*$ is varied into another vector $\mathfrak{y}$ of $\mathfrak{S}$ by setting

$$\mathfrak{y}(u', v') = \mathfrak{x}(u, v).$$

Substituting in the Dirichlet integral u', v' from (1) we obtain easily[8]

$$D(\mathfrak{y}) = D(\mathfrak{x}) + \epsilon V(\mathfrak{x}, \lambda, \mu) + \epsilon^2 R \tag{2}$$

where the first variation V of D is defined by

$$V(\mathfrak{x}, \lambda, \mu) = {}^1/_2 \int\int_B [p(\lambda_u - \mu_v) - q(\lambda_v + \mu_u)]dudv, \tag{3}$$

$$p = \mathfrak{x}_u{}^2 - \mathfrak{x}_v{}^2, \qquad q = -2\mathfrak{x}_u\mathfrak{x}_v,$$

and where

$$|R| \leq cD(\mathfrak{x}), \tag{4}$$

the constant c depending only on the bound a for the derivatives of Λ.

If $\mathfrak{x}$ is in $\mathfrak{P}$, then $p + iq = \varphi(w)$ is an analytic function of $w = u + iv$ in B, and $\varphi(w) = 0$ characterizes $\mathfrak{x}$ as a minimal surface.

By our transformation (1) the system (U) is taken into another system (U') of values $u_1', \ldots, u_N'$. In general, (1) will affect the points $u = \infty$, $u = -1$ and $u = 0$, so that vectors of $\mathfrak{S}$ or $\mathfrak{P}$ are transformed into vectors of $\mathfrak{S}^*$, $\mathfrak{P}^*$ no longer satisfying our three-point condition. However, by a linear transformation of w with real coefficients superimposed on (1), which leaves the Dirichlet integral and B invariant, we can always return to a vector of $\mathfrak{S}$ or $\mathfrak{P}$.

We say that $D(\mathfrak{x})$ is *stationary* if, first, $\mathfrak{x}$ is harmonic, and second, the first variation V vanishes for all functions λ, μ or Λ, M admitted in (1). Our remark then shows that for the stationary character of a vector $\mathfrak{x}$ in $\mathfrak{S}^*$ it suffices to prove $V = 0$ for all λ, μ or Λ, M for which Λ vanishes at $u = -1$, $u = 0$, $u = \infty$, so that for $\mathfrak{x}$ in $\mathfrak{S}$ also $\mathfrak{y}$ is in $\mathfrak{S}$. Unless stated otherwise we shall make this assumption throughout this paper.

2. *The Minimal Vector $\mathfrak{z}$ Is Uniquely Determined by (U).*—This follows from the essential fact[9] that for two vectors $\mathfrak{z}_1$ and $\mathfrak{z}_2$ belonging to (U), the linear combination $\mathfrak{x} = t\mathfrak{z}_1 + (1 - t)\mathfrak{z}_2$ for any t between 0 and 1 also belongs to (U). Setting $D(\mathfrak{z}_i) = d_i$ we have, by Schwarz's inequality, $D(\mathfrak{x}) \leq t^2 d_1 + (1 - t)^2 d_2 + 2t(1 - t)\sqrt{d_1 d_2}$, the equality sign holding only for $\mathfrak{z}_1 = \mathfrak{z}_2$. Hence, from $d_1 = d_2 = d$ and $\mathfrak{z}_1 \neq \mathfrak{z}_2$, it would follow that $D(\mathfrak{x}) < d$, contrary to the assumed minimum property of $d = d(U)$. Consequently our statement is proved; the space $\mathfrak{M}$ consists of an N-parametric family of vectors $\mathfrak{z}$.

3. *Any Admissible Vector $\mathfrak{x}$ Which Represents a Minimal Surface Belongs to $\mathfrak{M}$.*—In other words, simply-connected minimal surfaces bounded by Γ must be in the N-parametric family $\mathfrak{M}$.

Proof.—According to the principle of reflection for minimal surfaces,[10] on each open segment S_i: $u_i < u < u_{i+1}$ of C the vector $\mathfrak{x}$ is regular harmonic, the first component having a vanishing derivative with respect to v, the other components being constant (the components taken with respect to proper orthogonal axes.) For another admissible vector $\mathfrak{x} + \mathfrak{y}$ belonging to the same set (U) the components of $\mathfrak{y}$, except the first, vanish on S_i, and we therefore have on S_i everywhere $\mathfrak{y}\mathfrak{x}_v = 0$.

To characterize $\mathfrak{x}$ as a member of $\mathfrak{M}$ we have to show that $D(\mathfrak{x} + \mathfrak{y}) = D(\mathfrak{x}) + D(\mathfrak{y}) + 2D(\mathfrak{x}, \mathfrak{y}) > D(\mathfrak{x})$ which follows from $D(\mathfrak{x}, \mathfrak{y}) = 0$. This latter relation is obtained in the usual way by integrating first over B omitting small half-circles around the points of (U). Green's formula, because of $\Delta\mathfrak{x} = 0$ and $\mathfrak{y}\mathfrak{x}_v = 0$ on S_i, leaves only contributions from the circular arcs; and these contributions tend to zero if the radii of the small circles tend to zero through properly chosen sequences.

4. *The Function $d(U)$ Is Continuous.—Proof.*—In (2) and (4) we substitute for $\mathfrak{x}$ the minimalvector $\mathfrak{x} = \mathfrak{z}$ belonging to (U). If $\mathfrak{z}'$ denotes the minimalvector belonging to the varied set (U') we have $d' = d(U') = D(\mathfrak{z}') \leq D(\mathfrak{y})$, and therefore

$$d(U') \leq d(U) + \epsilon V(\mathfrak{z}, \lambda, \mu) + \epsilon^2 c d(U), \tag{5}$$

or, because of $|V(\mathfrak{x}, \lambda, \mu)| \leq 4aD(\mathfrak{x})$

$$d(U') \leq d(U)(1 + \epsilon 4a + \epsilon^2 c). \tag{6}$$

Since a similar inequality holds for the opposite transformation from (U') to (U) the continuity is proved.

5. The Minimizing Vector $\mathfrak{z}$ Depends Continuously on (U).—Proof.— Let $\epsilon = \epsilon_n$ be a sequence tending to zero, $\mathfrak{z}' = \mathfrak{z}_n$ the corresponding minimal vectors, and d_n the minimal values. Because of $d_n \to d$ the values d_n are bounded, hence the vectors $\mathfrak{z}_n$ belong to a compact space; they define, as the limit of a subsequence, a vector $\mathfrak{y}$ of $\mathfrak{P}$ which belongs to (U); because of the semicontinuity of $D(\mathfrak{x})$ we have $D(\mathfrak{y}) \leq \lim d_n = d$, hence $D(\mathfrak{y}) = d$. Consequently, since $\mathfrak{z}$ is uniquely determined by (U), $\mathfrak{y} = \mathfrak{z}$ is *the* minimal vector for (U) and the limit of the sequence $\mathfrak{z}_n$.

6. The Function $d(U)$ Possesses Continuous Derivatives with Respect to u_i.—Proof.[11]—The transformation inverse to (1), where $-\Lambda$, $-\mathrm{M}$ play the previous rôle of Λ, M, lead to the inequality

$$d = d(U) \leq d(U') - \epsilon V'(\mathfrak{z}', \lambda, \mu) + \epsilon^2 c d(U') \tag{7}$$

with

$$V'(\mathfrak{z}', \lambda, \mu) = {}^1/_2 \int\int_B [p'(\lambda_u - \mu_v) - q'(\lambda_v + \mu_u)] du dv \tag{8}$$

where we write u, v as independent variables instead of u', v' and where $p' = \mathfrak{z}_u'^2 - \mathfrak{z}_v'^2$, $\quad q' = -2\mathfrak{z}_u'\mathfrak{z}_v'$.

From (5) it appears immediately that $d(U')$ is bounded in ϵ for sufficiently small $|\epsilon|$. Furthermore, by using the result of sections 4 and 5 it is easily proved[12] that

$$V'(\mathfrak{z}', \lambda, \mu) \to V(\mathfrak{z}, \lambda, \mu) \tag{9}$$

as $\epsilon \to 0$. For $\epsilon > 0$ we infer from (5) and (7) that

$$V'(\mathfrak{z}', \lambda, \mu) - \epsilon c d(U') \leq \frac{d(U') - d(U)}{\epsilon} \leq V(\mathfrak{z}, \lambda, \mu) + \epsilon c d(U)$$

and hence, by (9), for $\epsilon \to 0$

$$\frac{d(U') - d(U)}{\epsilon} \to V(\mathfrak{z}, \lambda, \mu), \tag{10}$$

which shows that d possesses the derivative $V(\mathfrak{z}, \lambda, \mu)$ with respect to ϵ. Since d and d' depend only on (U) and (U'), we see: *The variation $V(\mathfrak{z}, \lambda, \mu)$ for a minimal vector depends only on the values of λ in the points of (U)*[13] *and is simply a linear combination of the derivatives of $d(U)$:*

$$V(\mathfrak{z}, \lambda, \mu) = \sum_1^N \alpha_i \frac{\partial d(U)}{\partial u_i} \tag{11}$$

where the α_i are constants depending on the values $\lambda(u_i)$. The derivatives $\dfrac{\partial d(U)}{\partial u_i}$ are obtained explicitly if we choose the variation (1) as a piece-

wise linear transformation which leaves $v' = v$ unchanged, takes one of the values u_i, e.g., u_2 into $u_2 + \epsilon$, and does not affect the others:

$$u' = u_1 + (u - u_1)\left(\frac{\epsilon}{u_2 - u_1} + 1\right) \text{ for } u_1 \leq u \leq u_2$$

$$u' = u_3 + (u - u_3)\left(\frac{\epsilon}{u_2 - u_3} + 1\right) \text{ for } u_2 \leq u \leq u_3$$

$$u' = u \text{ everywhere else.}$$

$\lambda = \Lambda$ is here given by $-\dfrac{u - u_1}{u_2 - u_1}, -\dfrac{u - u_3}{u_2 - u_3}$, respectively, and $\mu = \mathrm{M}$ is zero. Formula (10) then yields

$$\frac{\partial d(u_1, \ldots, u_N)}{\partial u_2} = -\int_0^\infty dv \left\{ \int_{u_1}^{u_2} \frac{\mathfrak{z}_u^2 - \mathfrak{z}_v^2}{u_2 - u_1}\, du + \int_{u_2}^{u_3} \frac{\mathfrak{z}_u^2 - \mathfrak{z}_v^2}{u_2 - u_3}\, du \right\} \tag{12}$$

which shows that the derivatives are continuous functions of the values (U).

7. *The Variational Problem for $D(\mathfrak{x})$ Is Equivalent to That of the Critical Values of $d(U)$.*—The variational problem is to find vectors $\mathfrak{x}$ in $\mathfrak{S}$ for which $D(\mathfrak{x})$ is stationary according to the definition in section 1. We then state: (*a*) $D(\mathfrak{x})$ is stationary for minimal surfaces and only for those. "Stationary vectors $\mathfrak{x}$," therefore, according to section 3, lie in the N-parametric family of vectors $\mathfrak{x}$ forming the space $\mathfrak{M}$. (*b*) If for a set (U) the vector $\mathfrak{x}$ represents a minimal surface then $d(U)$ is stationary in $u_1, \ldots, u_N$ at this set (U). (*c*) If for a set (U) the function $d(U)$ is stationary, then the corresponding $\mathfrak{x} = \mathfrak{z}$ makes $D(\mathfrak{x})$ stationary and hence represents a minimal surface. These three assertions contain the equivalence to be proved. Now (*a*) follows in exactly the same way as in the case of a relative minimum.[14] By substituting in $V = 0$ for μ identically zero and for λ a function which vanishes for large values of v and which for small values of v is the Poissonkernel $\dfrac{\omega - v}{(\omega - v)^2 + (\eta - u)^2}$ for the halfplane B and the point Q with the coordinates η and ω, we find that $q = -2\mathfrak{x}_u\mathfrak{x}_v$ is zero in Q and hence everywhere in B. Consequently the conjugate harmonic function $p = \mathfrak{x}_u^2 - \mathfrak{x}_v^2$ is constant in B, and this constant must be zero because $D(\mathfrak{x})$ exists; but $p + iq = 0$ characterizes $\mathfrak{x}$ as a minimalsurface. Conversely, for a vector $\mathfrak{x}$ of $\mathfrak{P}$ representing a minimal surface, we have $V = 0$ with arbitrary λ and μ.

To prove (*b*) we have only to notice that $V = 0$, for arbitrary λ and μ and for $\mathfrak{x} = \mathfrak{z}$ belonging to $\mathfrak{M}$, implies the vanishing of all derivatives of $d(U)$, according to section 5. To prove (*c*) we refer to formula (11)

which shows that $V(\mathfrak{z}, \lambda, \mu) = 0$ for arbitrary λ and μ if $d(U)$ is stationary; hence $D(\mathfrak{z})$ is stationary in the sense of the variational problem.

8. Concluding Remarks.—The reduction of the variational problem to a problem for a finite number of variables leads immediately to the conclusion: If two different relative minima for $D(\mathfrak{x})$ exist then there exists at least one stationary value of the type number one (minimax). Moreover, the general Morse relations for the stationary values of $D(\mathfrak{x})$ follow from the Morse theory of critical points for functions of N variables with continuous derivatives.

Since a maximum does not exist the type numbers in our finite problem cannot exceed $N - 1$. That our type numbers are identical with those defined for the variational problem by Morse is, incidentally, proved by a simple comparison.

It may be further remarked that the method and the result of this paper can be extended to the case of the general Plateau-Douglas problem for arbitrary topological structure if proper care is taken of possible degenerations.[15]

[1] Shiffman, "The Plateau Problem for Non-Relative Minima," *Ann. Math.*, **40** (1940) [1].

[2] Morse and Tompkins, "The Existence of Minimal Surfaces of General Critical Type," *Ann. Math.*, **40** (1940) [2].

[3] For relative minima the restriction to polygons can easily be removed by passing to a limit. For higher type numbers, however, this is not as simple. Therefore the result of this paper does not contain, and incidentally, is not contained in, the results of [1] or [2].

[4] Cf. Plateau's problem and Dirichlet's principle, *Ann. Math.*, **38** (1937) [3]; and, "The Existence of Minimal Surfaces under Given Boundary Conditions," *Acta Math.* **72**, 1940 [4]. Furthermore a note, "Remarks on the First Variation in Minimal Surface Theory," submitted to *Duke Jour.* in July 1940 [5].

[5] Cf., e.g. [4] p. 71.

[6] The construction of the solution proceeds in exactly the same way as for the Plateau problem, where (U) is not fixed. See e.g. [4] p. 72.

[7] See [4] p. 70.

[8] See [4] p. 60. In (2) we write $\mathfrak{v}(u,v)$ instead of $\mathfrak{v}(u', v')$.

[9] This linearity of the subspace of $\mathfrak{S}$ or $\mathfrak{P}$ for fixed (U), as exploited in sections 2 and 3, is the key to our solution of the problem.

[10] As observed also by M. Shiffman, this principle could be presented more briefly than in the literature, e.g., as follows: Suppose that $\mathfrak{x}$ has the three harmonic components x, y, z and that on $S = S_1$ we have $x = y = 0$. By reflection x and y can be harmonically extended beyond S and are regular in a neighborhood G of any closed subsegment S^* of S. In G the derivatives of x and y are bounded and x_u, y_u tend uniformly to zero as for the point P with the coördinates u, v in G the ordinate v tends to zero. From $x_u x_v + y_u y_v + z_u z_v = 0$ and $x_u^2 + y_u^2 + z_u^2 = x_v^2 + y_v^2 + z_v^2$ in G we infer, for P tending to S^*, uniformly $z_u z_v \to 0$, and hence $z_v \to 0$, because of $x_u \to 0$, $y_u \to 0$ and $z_u^2 \geq z_v^2 - x_u^2 - y_u^2$. Consequently the harmonic function $z(u, v)$ can be extended by reflection beyond S^*, and we have $z_v = 0$ on S^*.

[11] See the parallel argument in [5].

[13] See the corresponding proof in [5].

[13] This can be explicitly confirmed by realizing that on S_t we have $\delta_u^2 - \delta_v^2 = 0$, while this expression, except for minimal surfaces, is singular in the points of (U).

[14] See e.g. [3] or [4]. At this point a variation (1) in $\mathfrak{S}$ violating the three point condition is necessary so that the remark at the end of Section 1 is essentially needed for the proof; for the same reason it is not feasible to simplify our argument by assuming M = 0 throughout.

[15] See a forthcoming paper by M. Shiffman, submitted to the *Ann. Math.*

A NOTE ON CONFORMAL GEOMETRY

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I. Introduction.—In this note, we shall consider two Riemannian spaces of n-dimensions V_n, $'V_n$. The two spaces are said to be conformally related when corresponding to a coördinate system ξ^λ ($\lambda, \mu = 1 \ldots n$) in V_n, there exists a coördinate system ξ^λ ($\lambda, \mu = 1 \ldots n$) in $'V_n$ such that at corresponding points $P(\xi^\lambda)$ and $'P(\xi^\lambda)$, the metric tensors $a_{\lambda\mu}$, $'a_{\lambda\mu}$ of these spaces are related by

$$'a_{\lambda\mu} = \sigma a_{\lambda\mu}, \qquad \sigma = \sigma(\xi^\lambda). \tag{1.1}$$

From (1.1), it follows that the connections of these spaces

$$\Gamma^\kappa_{\mu\lambda} = \frac{a^{\kappa\nu}}{2}(\partial_\mu a_{\nu\lambda} + \partial_\lambda a_{\nu\mu} - \partial_\nu a_{\mu\lambda}), \partial_\nu = \frac{\partial}{\partial\xi^\nu}, \tag{1.2}$$

(and a similar equation for $'\Gamma^\kappa_{\mu\lambda}$) are related by

$$'\Gamma^\kappa_{\mu\lambda} = \Gamma^\kappa_{\mu\lambda} + A^\kappa_{(\mu} S_{\lambda)} - \tfrac{1}{2} a_{\mu\lambda} S^\kappa. \tag{1.3}$$

In (1.3), A^κ_μ is the unit affinor; the parenthesis $(\mu\lambda)$ indicates that the symmetric product is to be formed; and

$$S_\lambda = \partial_\lambda \log \sigma, \qquad S^\kappa = a^{\kappa\nu} S_\nu. \tag{1.4}$$

Our problem can be stated as follows: *If coördinate systems in $V_n(\xi^\lambda)$ and $'V_n(\xi^\lambda)$ can be chosen so that at corresponding points $P(\xi^\lambda)$, $'P(\xi^\lambda)$, the connections of these spaces are related by (1.3), then are the spaces conformally related?*

II. A System of Partial Differential Equations and Their Integrability Conditions.—From (1.2), we find

$$\partial_\lambda {}'a_{\sigma\mu} = {}'a_{\kappa\sigma}\,'\Gamma^\kappa_{\mu\lambda} + {}'a_{\kappa\mu}\,'\Gamma^\kappa_{\sigma\lambda}. \tag{2.1}$$

By substituting (1.3) into (2.1), the latter equation becomes

$$\partial_\lambda {}'a_{\sigma\mu} = {}'a_{\kappa\sigma}(\Gamma^\kappa_{\mu\lambda} + A^\kappa_{(\mu}S_{\lambda)} - \tfrac{1}{2}a_{\mu\lambda}S^\kappa) + {}'a_{\kappa\mu}(\Gamma^\kappa_{\sigma\lambda} + A^\kappa_{(\sigma}S_{\lambda)} - \tfrac{1}{2}a_{\sigma\lambda}S^\kappa). \tag{2.2}$$

From the standpoint of the system of partial differential equations (2.2), our problem becomes: *Given a gradiant vector S_λ and a symmetric tensor $a_{\lambda\mu}$ (and hence $\Gamma^\kappa_{\mu\lambda}$ can be formed by 1.2), then is the only solution of the above system of linear partial differential equations given by ${}'a_{\lambda\mu} = \sigma a_{\lambda\mu}$?*

Substituting $\sigma a_{\lambda\mu}$ for ${}'a_{\lambda\mu}$ in (2.2), we see that the latter equation is satisfied provided σ is a solution of (1.4) (and hence determined to within an arbitrary constant). However, the general solution of a system of partial differential equations in $\frac{n(n+1)}{2}$ variables ${}'a_{\lambda\mu}$ is given by

$${}'a_{\lambda\mu} = \underset{1}{C}\,\sigma a_{\lambda\mu} + \underset{2}{C}\overset{2}{a}_{\lambda\mu} + \ldots \underset{k}{C}\overset{k}{a}_{\lambda\mu},\ k = n(n+1)/2, \tag{2.3}$$

where $a_{\lambda\mu}$ (superindex $2 \ldots k$) are particular integrals of (2.2) and C (subindex $1 \ldots k$) are arbitrary constants.

By analyzing the integrability conditions of (2.2), we shall now show that they are satisfied if and only if ${}'a_{\lambda\mu} = \alpha a_{\lambda\mu}$ where α is a function of position. Since α must satisfy (1.4), it may be considered as coinciding with σ. Hence ${}'a_{\lambda\mu} = \sigma a_{\lambda\mu}$ will be shown to be the only solution of (2.2) which is compatible with the algebraic integrability conditions of (2.2).

By use of the covariant differentiation operator ∇_μ of V_n, (2.2) can be written as

$$\nabla_\lambda {}'a_{\sigma\mu} = {}'a_{\kappa\sigma}(A^\kappa_{(\mu}S_{\lambda)} - \tfrac{1}{2}a_{\mu\lambda}S^\kappa) + {}'a_{\kappa\mu}(A^\kappa_{(\sigma}S_{\lambda)} - \tfrac{1}{2}a_{\sigma\lambda}S^\kappa). \tag{2.4}$$

By covariant differentiation and alternation, we obtain as the integrability conditions of (2.4),

$$2\nabla_{[\alpha}\nabla_{\lambda]} {}'a_{\sigma\mu} = -K_{\alpha\lambda\sigma.}{}^{\nu}\,{}'a_{\nu\mu} - K_{\alpha\lambda\mu.}{}^{\nu}\,{}'a_{\nu\sigma}, \tag{2.5}$$

where $K_{\alpha\lambda\sigma.}{}^{\nu}$ is the Riemann-Christoffel tensor. From (2.4), we find

$$2\nabla_{[\alpha}\nabla_{\lambda]} {}'a_{\sigma\mu} = {}'a_{\kappa\sigma}(A^\kappa_{[\lambda}\nabla_{\alpha]}S_\mu - a_{\mu[\lambda}\nabla_{\alpha]}S^\kappa) + {}'a_{\kappa\mu}(A^\kappa_{[\lambda}\nabla_{\alpha]}S_\sigma - a_{\sigma[\lambda}\nabla_{\alpha]}S^\kappa). \tag{2.6}$$

Equating the right hand sides of (2.5) and (2.6), we obtain

$$-K_{\alpha\lambda\sigma.}{}^{\nu}\,{}'a_{\nu\mu} - K_{\alpha\lambda\mu.}{}^{\nu}\,{}'a_{\nu\sigma} = {}'a_{\sigma[\lambda}\nabla_{\alpha]}S_\mu - {}'a_{\kappa\sigma}a_{\mu[\lambda}\nabla_{\alpha]}S^\kappa + {}'a_{\mu[\lambda}\nabla_{\alpha]}S_\sigma - {}'a_{\kappa\mu}a_{\sigma[\lambda}\nabla_{\alpha]}S^\kappa. \tag{2.7}$$

From (1.3), we obtain

$${}'K_{\nu\mu\lambda.}{}^{\kappa} = K_{\nu\mu\lambda.}{}^{\kappa} + \frac{a^{\sigma\kappa}}{2}(a_{\lambda[\nu}S_{\mu]\sigma} - a_{\sigma[\nu}S_{\mu]\lambda}), \tag{2.8}$$

where

$$S_{\mu\lambda} = 2\nabla_{\mu}S_{\lambda} - S_{\mu}S_{\lambda} + \tfrac{1}{2}\, a_{\mu\lambda}S_{\rho}S^{\rho} = S_{\lambda\mu}. \tag{2.9}$$

Substituting (2.8) into (2.7) and using the fact that the Riemann-Christoffel tensor is alternating in the last two indices, (2.7) becomes

$$\begin{aligned}(a_{\sigma[\alpha}S_{\lambda]\beta} - a_{\beta[\alpha}S_{\lambda]\sigma})\, a^{\beta\nu\prime}a_{\nu\mu} + (a_{\mu[\alpha}S_{\lambda]\beta} - a_{\beta[\alpha}S_{\lambda]\mu})\, a^{\beta\nu\prime}a_{\nu\sigma} = \\ 2'a_{\sigma[\lambda}\nabla_{\alpha]}S_{\mu} - 2'a_{\kappa\sigma}a_{\mu[\lambda}\nabla_{\alpha]}S^{\kappa} + 2'a_{\mu[\lambda}\nabla_{\alpha]}S_{\sigma} - 2'a_{\kappa\mu}a_{\sigma[\lambda}\nabla_{\alpha]}S^{\kappa}.\end{aligned} \tag{2.10}$$

Upon replacing $S_{\mu\lambda}$ by its equivalent as given by (2.9), we obtain

$$\begin{aligned}a_{\sigma[\alpha}\,(2\nabla_{\lambda]}S_{\beta} - S_{\lambda]}S_{\beta} + \tfrac{1}{2}\, a_{\lambda]\beta}S_{\rho}S^{\rho})\, a^{\beta\nu\prime}a_{\nu\mu} - \\ a_{\beta[\alpha}\,(2\nabla_{\lambda]}S_{\sigma} - S_{\lambda]}S_{\sigma} + \tfrac{1}{2}\, a_{\lambda]\sigma}S_{\rho}S^{\rho})\, a^{\beta\nu\prime}a_{\nu\mu} + \\ a_{\mu[\alpha}\,(2\nabla_{\lambda]}S_{\beta} - S_{\lambda]}S_{\beta} + \tfrac{1}{2}\, a_{\lambda]\beta}S_{\rho}S^{\rho})\, a^{\beta\nu\prime}a_{\nu\sigma} - \\ a_{\beta[\alpha}\,(2\nabla_{\lambda]}S_{\mu} - S_{\lambda]}S_{\mu} + \tfrac{1}{2}\, a_{\lambda]\mu}S_{\rho}S^{\rho})\, a^{\beta\nu\prime}a_{\nu\sigma} = \\ 2'a_{\sigma[\lambda}\nabla_{\alpha]}S_{\mu} - 2'a_{\kappa\sigma}a_{\mu[\lambda}\nabla_{\alpha]}S^{\kappa} + 2\, a_{\mu[\lambda}\nabla_{\alpha]}S_{\sigma} - 2'a_{\kappa\mu}a_{\sigma[\lambda}\nabla_{\alpha]}S^{\kappa}.\end{aligned}$$

Cancelling the right-hand side terms against corresponding terms on the left-hand side and simplifying, we obtain for the integrability conditions of (2.2)

$$\begin{aligned}- a_{\sigma[\alpha}S_{\lambda]}S^{\nu\prime}a_{\nu\mu} - a_{\mu[\alpha}S_{\lambda]}S^{\nu\prime}a_{\nu\sigma} + {}'a_{\mu[\alpha}S_{\lambda]}S_{\sigma} + {}'a_{\sigma[\alpha}S_{\lambda]}S_{\mu} + \\ S_{\rho}S^{\rho}(a_{\sigma[\alpha}{}'a_{\lambda]\mu} + a_{\mu[\alpha}{}'a_{\lambda]\sigma}) = 0.\end{aligned} \tag{2.12}$$

Upon substituting into (2.12)

$${}'a_{\lambda\mu} = \sigma a_{\lambda\mu}, \tag{2.13}$$

we see that (2.12) is identically satisfied. This proves the sufficiency of (2.13). We now show that (2.13) is also necessary in order that (2.12) be valid.

Let us denote the n-mutually orthogonal unit vectors of V_n in the principal directions (assuming that they exist) of ${}'a_{\lambda\mu}$ by

$$\overset{i}{\underset{l}{}}{}^{\lambda} \qquad (l, k = 1 \ldots n). \tag{2.14}$$

Then we may write

$$a_{\lambda\mu} = \sum_{k} \overset{}{\underset{k}{}}{i_{\lambda}}\overset{}{\underset{k}{}}{i_{\mu}}, \tag{2.15}$$

$${}'a_{\lambda\mu} = \sum_{k} \overset{a}{\underset{k}{}}{i_{\lambda}}\overset{}{\underset{k}{}}{i_{\mu}}, \tag{2.16}$$

$$S_{\lambda} = \sum_{k} \overset{S}{\underset{k}{}}{i_{\lambda}}. \tag{2.17}$$

Now by transvecting (2.12) with $\overset{\sigma}{\underset{j}{}}\overset{}{\underset{l}{}}\overset{\alpha}{\underset{n}{}}\overset{\lambda}{\underset{q}{}}\overset{\mu}{}$ and using (2.15) to 2.17) we find

$$\begin{aligned}&(\delta S - \delta S)Sa + (\delta S - \delta S)Sa + \\ &\underset{jnl}{} \quad \underset{ljn}{} \quad \underset{qq}{} \quad \underset{qnl}{} \quad \underset{qln}{} \quad \underset{jj}{} \\ &(\delta S - \delta S)Sa + (\delta S - \delta S)Sa + \\ &\underset{qln}{} \quad \underset{qnl}{} \quad \underset{jq}{} \quad \underset{jln}{} \quad \underset{jnl}{} \quad \underset{qj}{} \\ &S^{\rho}S_{\rho}(\delta\delta a - \delta\delta a + \delta\delta a - \delta\delta a) = 0,\\ &\quad\underset{jlqnn}{} \quad \underset{jnlq}{} \quad \underset{qlnjn}{} \quad \underset{qnljl}{}\end{aligned} \tag{2.18}$$

where δ (subindex $l, j \ldots$) is the Kronecker symbol. If all the indices are distinct, then the left hand side of (2.18) vanishes; if $l = j$ and the remaining indices are distinct, we obtain

$$-\underset{nqq}{SSa} + \underset{nqj}{SSa} = 0. \tag{2.19}$$

Since S_λ is a non-zero vector, then S (subindex n) is not zero for some n and hence (2.19) becomes

$$-\underset{qq}{Sa} + \underset{qj}{Sa} = 0. \tag{2.20}$$

Now let S (subindex $1 \ldots m$) be the zero components of S_λ; and let S (subindex $m + 1 \ldots n$) be the non-zero components of S_λ. Take the index q in the last set and then (2.20) yields

$$\underset{q}{a} = \underset{j}{a} = \alpha, \quad q \neq j, j = 1 \ldots n. \tag{2.21}$$

Equation (2.18) now becomes an identity. When the condition (2.21) is used in (2.16), we find

$$'a_{\lambda\mu} = \alpha a_{\lambda\mu}. \tag{2.22}$$

Hence, we have the

THEOREM. *If coördinate systems in $V_n(\xi^\lambda)$ and in $'V_n('\xi^\lambda)$ can be chosen so that at corresponding points $P(\xi^\lambda)$, $'P('\xi^\lambda)$, the connections of these spaces are related by (1.3) and if the principal directions of $'a_{\lambda\mu}$ exist in V_n, then the spaces are conformal.*

HOMOTOPY RELATIONS IN FIBRE SPACES

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If X is a topological space, B a metric space with metric function $\rho(b, b')$, and π a continuous map of X on all of B, we shall say that *X is a fibre space over B relative to π* if there exist a positive ϵ_0 and a continuous function ϕ as follows:

1. $\phi = \phi(x, b)$ is a point of X and is defined for all $x \in X$, $b \in B$ such that $\rho(\pi(x), b) < \epsilon_0$,
2. $\pi\phi(x, b) = b$ wherever ϕ is defined,
3. $\phi(x, \pi(x)) = x$.

The map π is called *the projection* $X \to B$, and the sets $\pi^{-1}(b)$ in X are called the *fibres*. We refer to ϕ as the slicing function; for, if $x_0 \in X$ is fixed and b ranges over the ϵ_0 neighborhood of $\pi(x_0)$, $\phi(x_0, b)$ is a point of $\pi^{-1}(b)$ near x_0, so that ϕ provides a section through x_0 of the fibres neighboring x_0 which is homeomorphic to the neighborhood of $\pi(x_0)$. The simultaneous continuity of ϕ in x, b insures that this section varies continuously with x. Note that ϕ is defined in a neighborhood U of the graph G of π in the product space $X \times B$, and the correspondence $(x, b) \to (\phi(x, b), b)$ is a retraction of U into G parallel to X. Conversely if such a retraction exists, the function ϕ is readily constructed. Observe also that π is an interior map (carries open sets into open sets). If we did not insist that $\pi(X)$ be all of B, it would follow that $\pi(X)$ is both open and closed in B.

The class of fibre spaces includes the class of fibre bundles in the sense of Whitney.[1] We shall need the following examples of fibre spaces which are also fibre bundles.

I. The product space $X = B \times A$.—Here $\pi(b, a) = b$, and $\phi((b, a), b') = (b', a)$; i.e., ϕ is defined over all of $X \times B$ and the slices are the sections parallel to the coördinate B.

II. X a covering space of B.—The function π attaches to each point of X the point of B it covers. It is supposed there is an $\epsilon_0 > 0$ such that π is a topological map on a neighborhood of x whose image contains all points at a distance $< \epsilon_0$ from $\pi(x)$. Then ϕ attaches to (x, b) that point in the neighborhood of x whose image is b.

III. $\pi(X) = B$ *a non-singular map of one differentiable manifold on another.*—By non-singular we mean that the Jacobian of π has maximum rank at every point of X. Then the fibres are differentiable submanifolds. The function ϕ is defined by introducing a Riemannian geometry in X and sectioning the fibres by perpendicular geodesic planes. An example of this is the Hopf[2] mapping of the 3-sphere on the 2-sphere ($S^3 \to S^2$), likewise his mappings $S^7 \to S^4$ and $S^{15} \to S^8$. The fibres are great spheres of dimensions 1, 3 and 7, respectively.

IV. B a coset space of the compact Lie group X.—We consider a closed subgroup H of X, and define B to be the space of left (or right) cosets, and $\pi(x)$ = the coset containing x. We first define $\phi(1, b)$ by means of a coordinate neighborhood of the unit 1 and a plane perpendicular to the tangent plane of H at 1. Then $\phi(x, b) = x\phi(1, x^{-1}b)$. For example, let R_n be the rotation group of the n-sphere S^n. If $b_0 \in S^n$ is fixed and $\pi(r) = r(b_0)$, this projection of R_n in S^n is the projection of R_n into the space of

left cosets of the subgroup R_{n-1} leaving b_0 fixed. In this way R_n is a fibre space over S^n.

Our principal tool is embodied in[3]

THEOREM 1. *If X is a fibre space over B, Y a topological space, g a continuous map of Y in X, and $h(y, t)$ $(0 \leqq t \leqq 1)$ a homotopy of the map $h(y, 0) = \pi g(y)$ uniform in the sense that there is a $\delta_0 > 0$ such that $|t - t'| < \delta_0$ implies $\rho(h(y, t), h(y, t')) < \epsilon_0$ for all $y \in Y$, then there exists a homotopy $g(y, t)$ of g in X (called the covering homotopy) such that $\pi g(y, t) = h(y, t)$. In addition, if $h(y, t)$ leaves y fixed, so also does $g(y, t)$.*

Subdivide the interval (0, 1) into the subintervals (t_i, t_{i+1}) of length $< \delta_0$, and define $g(y, t)$ stepwise by $g(y, t) = \phi(g(y, t_i), h(y, t))$, $(t_i < t \leqq t_{i+1})$.

In case Y is compact metric, the uniformity requirement is redundant.

COROLLARY 1. *If B is arcwise connected, any two fibres have the same homotopy type.*

If F_1, F_2 are the fibres over b_1, b_2, respectively, and $h(t)$ is a path from b_1 to b_2. Choose $Y = F_1$, $g =$ identity and h as the homotopy of $\pi(F_1)$, then the covering homotopy deforms F_1 into F_2. Using $h'(t) = h(1 - t)$, we deform F_2 into F_1. Since $h'h$ is homotopic to b_1, the homotopy of F_1 into F_2 back into F_1 is homotopic to a homotopy in F_1. Similarly for F_2.

COROLLARY 2. *The Hopf mappings $S^3 \to S^2$, $S^7 \to S^4$, $S^{15} \to S^8$ are essential (i.e., the images are not homotopic to points).*

Let $Y = S^3$ and $g =$ identity. A contraction of $\pi(S^3)$ to a point b_0 of S^2 would be covered by a contraction of S^3 on itself into the circle over b_0. This is impossible since no homotopy of S^3 on itself can free a point.

In the following theorems, $\pi_i(X, F)$ is the ith homotopy group[4] of X relative to the closed set F. If F is a single point and X is arcwise connected, the group is independent of F and we write $\pi_i(X)$.

THEOREM 2. *If X is a fibre space over B, and F_0 is the fibre over the point b_0, then $\pi_i(X, F_0) = \pi_i(B, b_0)$.*

An element of $\pi_i(X, F_0)$ is represented by a continuous map f of an i-cell E^i carrying the boundary $\dot{E}^i$ into F_0 and a fixed point $y_0 \in \dot{E}^i$ into a fixed point $x_0 \in F_0$. Clearly πf represents an element of $\pi_i(B, b_0)$. This defines a homomorphism $\pi_i(X, F_0) \to \pi_i(B, b_0)$. If πf is homotopic to b_0 leaving $\dot{E}^i$ at b_0, the covering homotopy deforms $f(E^i)$ into F_0 keeping $\dot{E}^i$ in F_0 and y_0 at x_0. The correspondence is therefore an isomorphism into a subgroup. Let $g(E^i)$ represent an element of $\pi_i(B, b_0)$. If $h(y, t)$ contracts E^i on itself into y_0, then gh contracts $g(E^i)$ into $g'(E^i) = b_0$. Define $f'(E^i) = x_0$. Then $\pi f' = g'$, and the homotopy of g' into g is covered by a homotopy of f' into an f such that $\pi f = g$. Since gh leaves y_0 at b_0, we have $f(y_0) = x_0$ and the theorem is proved.

COROLLARY 3. *If X is a covering space of B, then $\pi_i(X) = \pi_i(B)$ for $i \geqq 2$.*

Since F_0 is a discrete set of points, and $\dot{E}^i$ is connected, any map of E^i representing an element of $\pi_i(X, F_0)$ must carry $\dot{E}^i$ into x_0. Hence $\pi_i(X, F_0) = \pi_i(X, x_0)$.

COROLLARY 4. *For the 1-sphere S^1, we have $\pi_i(S^1) = 0$ for $i \geqq 2$.*

The covering space of S^1 is a line L, and $\pi_i(L) = 0$.

THEOREM 3. *If S^n is an n-sphere and F a closed arcwise connected proper subset of S^n, then $\pi_i(S^n, F)$ is the direct sum $\pi_i(S^n) + \pi_{i-1}(F)$.*

Let $x_0 \in F$ be the fixed reference point and choose a fixed homotopy $h(x, t)$ of F in S^n contracting F into x_0 and leaving x_0 fixed. Choose a fixed map g of E^{i-1} on $\dot{E}^i$ carrying $\dot{E}^{i-1}$ into y_0 and being topological on $E^{i-1} - \dot{E}^{i-1}$. If f represents an element of $\pi_i(S^n, F)$, then $f' = fg$ represents an element of $\pi_{i-1}(F)$. This defines a homomorphism $\pi_i(S^n, F) \to \pi_{i-1}(F)$. Using coordinates (y, r) in E^i where r = radius vector, and y is the central projection of the point on $\dot{E}^i$, we define

$$f''(y, r) = \begin{cases} f(y, 2r), & 0 \leqq r \leqq 1/2, \\ h(f(y, 1), 2r - 1), & 1/2 \leqq r \leqq 1, \end{cases}$$

and f'' represents an element of $\pi_i(S^n)$. This defines a homomorphism $\pi_i(S^n, F) \to \pi_i(S^n)$. These two homomorphisms induce a homomorphism into the direct sum. On the other hand if f' and f'' are given, we define

$$f(y, r) = \begin{cases} f''(y, 2r), & 0 \leqq r \leqq 1/2 \\ h(f'g^{-1}(y), 2 - 2r), & 1/2 \leqq r \leqq 1; \end{cases}$$

and this shows that the correspondence with the direct sum is 1–1.

Theorems 2 and 3 together with the Hopf mappings of spheres on spheres give the following results.

THEOREM 4. $\pi_i(S^2) = \pi_i(S^3) + \pi_{i-1}(S^1)$, $\pi_i(S^4) = \pi_i(S^7) + \pi_{i-1}(S^3)$, $\pi_i(S^8) = \pi_i(S^{15}) + \pi_{i-1}(S^7)$.

The first of these relations and Corollary 4 give

COROLLARY 5. $\pi_i(S^2) = \pi_i(S^3)$ *for* $i \geqq 3$.

Since $\pi_i(S^n) = 0$ for $i < n$, and $\pi_n(S^n)$ is the infinite cyclic group, denoted by ∞, we have

COROLLARY 6. $\pi_i(S^4) = \pi_{i-1}(S^3)$ for $i = 2, \ldots, 6$, and $\pi_7(S^4) = \infty + \pi_6(S^3)$, $\pi_i(S^8) = \pi_{i-1}(S^7)$ for $i = 1, \ldots, 14$, and $\pi_{15}(S^8) = \infty + \pi_{14}(S^7)$.

The first parts of these relations are special cases of some results of Freudenthal[5] (obtained in an entirely different manner) which state: $\pi_i(S^n) = \pi_{i-1}(S^{n-1})$ for $i = 2, \ldots, 2n-2$ and $n = 2, 3, \ldots$.

The results of the following theorem are based on the coset mapping $R_n \to S^n$ described in IV.

THEOREM 5. $\pi_i(R_{i+1})$ is a factor group of $\pi_i(R_i)$, and $\pi_i(R_{i+1}) = \pi_i(R_{i+k})$ for $k, i = 1, 2, \ldots$.

Since $\pi_i(S^n) = 0$ for $i < n$, any continuous map of an i-sphere in S^n is

homotopically deformable into the point b_0. If this i-sphere is mapped in S^n through R_n, the covering homotopy deforms the i-sphere into R_{n-1}. Hence any i-sphere in R_n, $i < n$, is deformable into R_i. If an i-sphere in R_i is the boundary of an $(i+1)$-cell in R_n, the image of this $(i+1)$-cell in S^n is contractible into b_0 leaving the boundary of the cell at b_0, providing $i + 1 < n$. Hence if an i-sphere of R_i is contractible in R_n $(n > i)$, it is contractible in R_{i+1}).

COROLLARY 7. $\pi_1(R_n)$ = the cyclic group of period 2 for $n > 1$, and $\pi_2(R_n) = 0$ for $n > 0$.

Since R_2 = projective 3-space, $\pi_1(R_2)$ = group of period 2. Since R_2 is covered by S^3 and $\pi_2(S^3) = 0$, we have by Corollary 3 that $\pi_2(R_2) = 0$.

The results of Theorem 4 cast considerable light on the problem of finding fibre mappings of spheres on spheres. If S^k is a fibre space over $S^n (k \geqq n)$ with fibre F, then $\pi_i(S^n) = \pi_i(S^k) + \pi_{i-1}(F)$. This implies $\pi_{i-1}(F) = 0$ for $i < n$. Hence if the dimension of F is $\leqq n - 2$, F is contractible on itself to a point. This implies $\pi_i(F) = 0$ for all i, and therefore $\pi_i(S^n) = \pi_i(S^k)$. This can happen only if $k = n$. On the other hand, if the dimension of F is $> n - 2$, we obtain $k \geqq 2n - 1$. Thus S^{2n-1} is the sphere of least dimension which can be a proper fibre space over S^n. If we require in addition that the fibre F be a sphere, we obtain from $\pi_{n-1}(F) = \pi_n(S^n)$ that $F = S^{n-1}$. Hence only S^{2n-1} can be proper a sphere space over S^n.

The following is an example of a fibre space which is not a fibre bundle in the sense of Whitney. Let B = the interval $0 \leqq x \leqq 1$ and let X = the triangle in the (x, y)-plane defined by $0 \leqq y \leqq x \leqq 1$. Define $\pi(x, y) = x$. The slicing function ϕ is then given by

$$\phi((x, y), x') = \begin{cases} (x', x') \text{ for } x' \leqq y \\ (x', y) \text{ for } x' > y. \end{cases}$$

[1] These PROCEEDINGS, **26**, 148–153 (1940); also **21**, 464–468 (1935).

[2] *Fundamenta Math.*, **25**, 427–440 (1935).

[3] An inverse of this theorem has been proved by Dr. R. H. Fox as follows: If B is an absolute neighborhood retract and $\pi(X) = B$ is continuous and there is an $\epsilon_0 > 0$ such that the conclusions of Theorem 1 hold, then X is a fibre space over B relative to π.

[4] See W. Hurewicz, *Proc. Amsterdam Acad.*, **38**, 112, 521 (1935); also **39**, 117, 215 (1936).

[5] *Compositio Math.*, **5**, 299–314 (1937).

NOTE ON LINEAR DIFFERENCE AND DIFFERENTIAL EQUATIONS

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The modern analytic theory of ordinary linear difference equations had its inception in an early paper of Henri Poincaré published in 1885. More than two decades later the work of N. E. Nörlund, R. D. Carmichael and myself led to what, in a certain limited sense, is a "general" theory. More recently the existence of a full complement of formal solutions has been established in all cases, and on this basis W. J. Trjitzinsky and I have given jointly a truly general theory.[1]

Much before this latest work, the very clear conviction had come to me that the analytic theory of linear difference equations provided a complete methodological pattern in the formulation of a comparable general theory for the essentially simpler but analogous field of linear differential equations. This point of view was altogether substantiated by Trjitzinsky in his subsequent development of the general theory of linear differential equations.[2]

What I desire to point out in the present Note is that the methodological relationship between the two fields is so close that the theory of linear differential equations falls out as an immediate corollary of the theory of linear difference equations. For the sake of simplicity, however, I shall establish this fact only for the typical case of a linear differential system (in matrix notation)

$$Y'(x) = A(x)Y(x) \tag{1}$$

with an irregular singular point of rank 0 at $x = \infty$, so that the nth order square matrix $A(x) \equiv (a_{ij}(x))$ is analytic at $x = \infty$. A graduate student at Harvard University, Mr. E. C. Gras, is undertaking to carry out systematically the extension of this program to the general case.

In the first place there is an immediately provable theorem[3] which allows us to conclude that any matrix solution $Y(x)$ of (1) satisfies a functional equation

$$Y(\varphi(x)) = M(x)Y(x)$$

where $M(x)$ is analytic at $x = \infty$, provided that $\varphi(x)$ is a function of the form (case $q = 0$ of the theorem)

$$\varphi(x) = x + l + \frac{m}{x} + \dots$$

For our purposes here it suffices merely to take

$$\varphi(x) = x + 1$$

and thus arrive at the difference equation

$$Y(x + 1) = M(x)Y(x) \tag{2}$$

where $M(x)$ is analytic at $x = \infty$.

Now in accordance with the general theory of the difference equation (2), there will exist a formal matrix solution $S(x)$ which has the form

$$S(x) \equiv (\rho_j^x x^{r_j} s_{ij}(x)) \tag{3}$$

in case the characteristic equation

$$|\rho\delta_{ij} - m_{ij}(\infty)| = 0$$

has n distinct roots $\rho_1, \ldots \rho_n$; here the expressions $s_{ij}(x)$ stand for descending formal power series in $1/x$ such that the determinant of the leading coefficients $|s_{ij}(\infty)|$ is not zero. We confine attention to this case.[4]

But the general theory in this non-singular case[5] shows that there will then be two special "principal solutions" $Y_+(x)$ and $Y_-(x)$ such that the asymptotic formulae hold:

$$\left.\begin{aligned} Y_+(x) &\sim S(x), -\frac{\pi}{2} - \epsilon \leqq arg\, x \leqq \frac{\pi}{2} + \epsilon, \\ Y_-(x) &\sim S(x), \frac{\pi}{2} - \epsilon \leqq arg\, x \leqq \frac{3\pi}{2} + \epsilon. \end{aligned}\right\} \tag{4}$$

The most general solution of (2) may be written as $Y_0(x)P(x)$ where $Y_0(x)$ is any particular matrix solution and $P(x)$ is a periodic matrix of period 1. Hence any solution $Y(x)$ of (1) may be expressed in the first one of the specified sectors as

$$Y(x) = Y_+(x)P(x) \tag{5}$$

Here $P(x)$ is not only periodic of period 1 but analytic in the finite plane with $|P(x)| \neq 0$, since $|Y(x)| \neq 0$ and $|Y_+(x)| \neq 0$, for $|x|$ sufficiently large in the right half plane. Substituting this expression in (1) we readily obtain

$$Y_+^{-1}(x)[Y_+'(x) - A(x)Y_+(x)] = P'(x)P^{-1}(x), \tag{6}$$

which leads at once to an asymptotic relationship in this sector

$$P'(x)P^{-1}(x) \sim ((\rho_j/\rho_i)^x x^{r_j - r_i} t_{ij}(x)). \tag{7}$$

The matrix $P'(x)P^{-1}(x)$ appearing here on the left is of course analytic in the finite plane and periodic of period 1. The expressions $t_{ij}(x)$ appear-

ing on the right stand for descending formal power series in $1/x$. The precise meaning of the asymptotic relationship written is obvious.

But if we transfer attention to the z-plane where

$$z = e^{2\pi i x},$$

this relationship shows that the typical element $q_{ij}(z)$ of the matrix on the left in (7) is not only single-valued and analytic for $z \neq 0, \infty$, but is of finite order at these excepted points. Thus $q_{ij}(z)$ must be polynomials in $1/z$ and z. The same relationship also shows this to be impossible unless $t_{ij}(x) \equiv 0$ for all i and j. It follows therefore from (7) that $S(x)$ is a formal matrix solution of the given differential equation (1), and that we may put $t_{ij}(x) \equiv 0$ in (7):

$$P'(x)P^{-1}(x) \sim ((\rho_{j/\rho i})^x(0)). \tag{8}$$

However it is then clear that the product $q_{ij}(z)q_{ij}(1/z)$ is not only analytic for $z \neq 0, \infty$, but vanishes at these points. Hence the matrix $P'(x)$-$P^{-1}(x)$ and so $P'(x)$ vanish identically. Consequently $P(x)$ is a constant matrix, of constants C; and, by (6), $Y_+(x)$ is a solution of the given differential equation (1), while $Y(x) = Y_+(x)C$ is the given solution of (1).

It is thus immediately established that the given linear differential system (1) admits of matrix solutions $Y(x)$ asymptotic to $S(x)$ in the two specified sectors covering the entire plane. From this follow at once all the usual facts about the asymptotic behavior of solutions in the complete neighborhood of $x = \infty$.

There can be no doubt that the intensification of this type of argument, based on the general theory of linear difference equations, will lead easily and directly to the asymptotic characterization of the solutions of linear differential equations at any regular or irregular singular point. Furthermore it is equally clear that in a similar manner the formal theory and the inverse Riemannian theory for differential equations follow directly from the corresponding results for difference equations.

[1] G. D. Birkhoff, "Formal Theory of Irregular Linear Difference Equations," *Acta Mathematica*, **54** (1930); G. D. Birkhoff and W. J. Trjitzinsky, "Analytic Theory of Singular Difference Equations," *ibid.*, **60**, 1–89 (1936).

[2] W. J. Trjitzinsky, "Analytic Theory of Linear Differential Equations," *ibid.*, **62**, 167–226 (1938).

[3] G. D. Birkhoff, "Theorem Concerning the Singular Points of Ordinary Differential Equations," these PROCEEDINGS, **1**, 578–581 (1915).

[4] If this restriction is not satisfied, more complicated types of series and a more complicated analytic situation may arise, but the general course of the argument is not affected.

[5] See, for instance, my paper, "General Theory of Linear Difference Equations," *Trans. Am. Math. Soc.*, **10**, 436–470 (1909).

MAXIMAL SUBGROUPS OF A GIVEN GROUP

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A maximal subgroup of a given group G is a proper subgroup of G, that is, a subgroup which is neither the identity nor G itself,[1] and in order to be maximal it cannot be contained in a larger proper subgroup of G. When every proper subgroup of G is maximal then every proper subgroup of G is also minimal, that is, it is of prime order, and vice versa, when every proper subgroup of G is minimal then every proper subgroup of G is also maximal. In each of these two cases G is one of the two possible groups of order p^2, p being a prime number, one of the two possible groups of order pq, p and q being distinct prime numbers such that $p - 1$ is divisible by q, or G is the cyclic group of order pq where $p - 1$ is not divisible by q and $q - 1$ is not divisible by p. If the order of a group is divisible by more than two prime numbers, equal or unequal, it is not possible for all of its proper subgroups to be either maximal or minimal since the Sylow subgroups could obviously not then be maximal when all of these prime numbers are distinct.

Suppose that G contains one and only one maximal subgroup. This subgroup must then be invariant and it must be generated by every operator of G which does not appear in it since every operator of G appears in at least one maximal subgroup of G. *The cyclic group of order p^m, p being an arbitrary prime number and $m > 1$, is the only group which contains one and only one maximal subgroup.* The determination of the maximal subgroups of the groups of order p^m is clearly equivalent to the determination of its subgroups of index p. In particular, the cross-cut of all such maximal subgroups is the ϕ-subgroup of the group and every non-cyclic group of order p^m contains at least $p + 1$ maximal subgroups. There is one and only one abelian group of order p^m which contains exactly $p + 1$ maximal subgroups and involves operators of order p^{m-1}, and when $m > 1$ there is also one and only one such non-abelian group, except when $m = 3$ and $p = 2$. In this special case there are two such non-abelian groups.

Let G be an abelian group which contains two and only two maximal subgroups. It was noted above that the order of G is then divisible by at least two distinct prime numbers and it can obviously not be divisible by more than two distinct prime numbers. Moreover, its two Sylow subgroups must be cyclic and it is the direct product of its two Sylow subgroups. Its ϕ-subgroup is the direct product of the subgroups of prime index in its Sylow subgroups. It was noted above that when these Sylow subgroups are of prime order then every maximal subgroup of G is also a

minimal subgroup of G. In this special case the ϕ-subgroup of G is the identity, and the ϕ-subgroup of G is always the cross-cut of its two maximal subgroups; this cross-cut is composed of all the operators of G whose orders are not divisible by at least one of the largest powers of the prime numbers which divide the order of G.

If any given group G contains two and only two maximal subgroups, each of them is invariant under G because an operator of one of these two subgroups could not transform the other into the one which contains this operator. The index of each of these subgroups under G is a prime number and their cross-cut is an invariant subgroup of G. The corresponding quotient group contains two and only two proper subgroups and hence it is the cyclic group of order pq, p and q being distinct prime numbers. One of the two given maximal subgroups therefore contains a Sylow subgroup whose order is a power of p while the other contains a Sylow subgroup whose order is a power of q. Each of these two Sylow subgroups is invariant under G since the quotient group of G with respect to the given invariant cross-cut contains only one subgroup of order p and also only one subgroup of order q.

As the Sylow subgroups of G whose orders are powers of p are conjugate under the given maximal subgroup of index q as well as under G, each of these Sylow subgroups is invariant under an operator whose order is a power of q contained in G. Similar remarks apply to the Sylow subgroups whose orders are powers of q which appear in one of the given maximal subgroups. Moreover, there can be only one such Sylow subgroup in each case since the quotient group with respect to the given cross-cut is the cyclic group of order pq. It therefore results that G contains the direct product of these two Sylow subgroups since they are both invariant and have only the identity in common. As G is assumed to contain two and only two maximal subgroups it has been proved that *the abelian groups which are the direct products of two prime power cyclic groups whose orders are powers of distinct prime numbers are the only groups which separately contain two and only two maximal subgroups.*

It has now been proved that whenever a group contains either exactly one maximal subgroup or exactly two maximal subgroups it must be abelian. When it contains exactly three maximal subgroups it is not necessarily abelian as results from the octic and the quaternion groups. Maximal subgroups constitute an important part in the theory of permutation groups in view of the fact that a necessary and sufficient condition that a transitive permutation group of degree n is primitive is that the subgroup composed of all its permutations which omit a given letter is maximal, and vice versa. In particular, every symmetric group of degree $n > 2$ is primitive and every such alternating group is also primitive. It is therefore possible to find at least two distinct groups of every degree

$n > 3$ which separately contain at least n maximal subgroups and are non-abelian.

Suppose that the group G contains exactly three maximal subgroups and that not all of them are invariant under G. They could not form a simple set of conjugates because they would then be transformed under G according to a permutation group of degree 3. This could not be the cyclic group of degree 3 because the three maximal subgroups could not be transformed cyclically by one of them. It could not be the symmetric group of degree 3 because this contains four maximal subgroups. It would not be possible for one of the three maximal subgroups to be invariant under G and the other two to be conjugate under G since the operators which are not common to these two conjugate subgroups would have to transform the remaining operators of the other among themselves. Hence it results that *when G contains exactly three maximal subgroups they are all invariant under G.*

It was noted above that every abelian group of order 2^m which has exactly two independent generators has also exactly three maximal subgroups and that these are the only abelian groups of order 2^m which separately have exactly three maximal subgroups. An infinite system of non-abelian groups of order 2^m which separately contain three and only three maximal subgroups was also noted above. The group of lowest order which contains three and only three maximal subgroups is the four-group, which also contains three minimal subgroups. While the maximal subgroups are of considerable interest the minimal subgroups are so very simple that it is somewhat questionable whether a special term should be used for them since the term groups of prime order is sufficiently descriptive.

If G is the group of order p^m, $m > 2$, which is obtained by extending the cyclic group of order p^{m-1} by an operator of order p which is commutative with the operators of its subgroup of index p but with no other operator of this subgroup of order p^{m-1} then G is non-abelian. When $p > 2$ it is conformal with the abelian group of type 1, $m - 1$, and this is also the case when $p = 2$ and $m > 3$. When $p = 2$ and $m = 3$ it is either the octic group or the quaternion group. In all cases when $m > 2$ it contains exactly $p + 1$ maximal subgroups and hence it contains exactly three such subgroups when $p = 2$. Each of these subgroups is abelian and when $m > 3$ two of them are cyclic while the third is non-cyclic. Another infinite system of groups composed of groups which separately have exactly three maximal subgroups is composed of the cyclic groups whose orders are the products of the powers of three distinct prime numbers.

When the order of G is not divisible by three distinct prime numbers then this order is of the form 2^m since it must then be generated by two of the three invariant maximal subgroups and the quotient group with respect to their cross-cut is then the four group. This results from the facts that

these maximal subgroups give rise to quotient groups of prime order and if this order would exceed 2 there would be more than three maximal subgroups in G. When the order of G is divisible by three distinct prime numbers it is evident that the given system of direct products of cyclic prime power groups includes all the possible cases in which each of the groups contains exactly three maximal subgroups. In this case all of these groups are therefore abelian.

[1] Sometimes the identity is included among the proper subgroups of a given group. In the *Enzyklopädie der Mathematischen Wissenschaften*, 1, 9, 7 (1939), the identity is called an actual (echte) subgroup but not a proper (eigentliche) subgroup.

A UNIFIED THEORY OF INTEGRATION

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The purpose of this note is to present a unified approach to the integrals of Riemann, Lebesgue, Stieltjes, Bochner and Denjoy. Integrable functions are defined as limits of series of step functions. The problem is to give, for each type of integration, a definition of convergence for series of step functions such that the class of limit functions will coincide with the class of integrable functions. Two types of extension are considered. The first depends on the metric completion of the space of step functions. This leads to the integrals of Lebesgue, Stieltjes and Bochner and may be used to construct the spaces L_p. The second method characterizes the class of measurable almost everywhere finite functions as limits of series of step functions and then proceeds, by transfinite induction to define an integral over a subclass of these functions. This yields the Denjoy integral. Both of these methods of extension depend directly on intervals and no use is made of open, closed or measurable sets. However, the theorems relating integration to such sets are readily accessible by these methods.

The first method derives its motivation from two sources. On the one hand, Riemann integration is a method of approximation by step functions while, on the other hand, it is well known that the Lebesgue summable functions form a complete, separable, metric space in which the rational step functions are dense. These facts suggest that a definition of convergence for series of step functions might be found which would include the Riemann type of approximation as a special case but which would be sufficiently general to complete the metric space of step functions and in-

clude as limit functions all Lebesgue summable functions. If this can be done, theorems involving summable functions can be proved first for step functions and then extended to summable functions by a single limit operation.

A satisfactory definition of convergence for series of step functions must fulfil the following four conditions.

1. Every convergent series of step functions must converge in a pointwise sense almost everywhere.
2. The integral series, obtained by term by term integration, of a convergent series of step functions must converge.
3. If two convergent series of step functions converge in the pointwise sense to the same function almost everywhere, then their integral series must converge to the same limit.
4. Every Lebesgue summable function must be the pointwise limit almost everywhere of a convergent series of step functions.

Under the Darboux definition of the Riemann integral, a sequence of step functions defined over a finite interval is said to be convergent if it is monotone and uniformly bounded. A Riemann integrable function can be approached either from above or from below by such a sequence. The first three conditions are met, the fourth is not.

By retaining the condition of uniform boundedness but replacing the requirement of monotonicity by condition one, Riesz[1] obtains a larger class of functions, namely, the bounded Lebesgue summable functions. A second extension is necessary to obtain the unbounded summable functions.

On the other hand, Young[2] drops the condition of uniform boundedness while retaining the requirement of monotonicity. He obtains the Lebesgue summable functions by two successive extensions of this type, one using increasing and one using decreasing sequences.

Under the Lebesgue method, measurable sets are obtained by an extension of the Young type in which the step functions are restricted to being characteristic functions, that is, assume only the values 0 and 1. This is followed by an extension of the Darboux type which yields the class of bounded summable functions and this, in turn, is followed by a further extension to obtain the unbounded summable functions.

None of these methods fulfils condition four on the first step. At least two successive extensions are required in every case and the extension of theorems from step functions to summable functions must be made in at least two steps. Furthermore, the salient fact that the extension is the completion of a metric space is lost sight of and must be established as a theorem.

In this note we shall deal with series rather than sequences of step functions. The type of convergence which we shall consider is absolute convergence. This method will be presented for the case of real valued func-

tions defined on the linear continuum and the Lebesgue integral is obtained. However, it can be applied without change to functions whose values lie in a Banach[3] space and are defined over a normed Boolean[4] ring. Thus integrals of the Bochner[5] and Stieltjes types may be obtained by this construction.

Let $u(x)$ denote a step function where $u = c_i$ on the interval $a_i \leq x < b_i$, where $a_i < b_i \leq a_{i+1}$ and $i = 1, 2, \ldots, n$. In the construction which follows the a_i, b_i and c_i may be restricted to rational values without loss of generality. If this is done the completed space is immediately seen to be separable.

DEFINITION 1. $\int u\,dx = \sum_{i=1}^{n} c_i(b_i - a_i)$, where the limits of integration are understood to be from $-\infty$ to $+\infty$.

DEFINITION 2. $||u|| = \int |u|\,dx$. $||u||$ is called the *variation* of $\int u\,dx$ and has the usual properties of an absolute value. The double bar is used to distinguish it from $|u|$ which is taken in a pointwise sense.

DEFINITION 3. A series $\sum_{n=1}^{\infty} u_n(x)$ is *absolutely convergent* if $\sum_{n=1}^{\infty} ||u_n(x)||$ converges.

The series associated with a bounded, monotone sequence of step functions clearly satisfies this definition so that it is evident that this definition includes the sequences used in defining the Riemann integral. On the other hand, absolute convergence is readily defined in any metric space. Moreover every convergent sequence in a metric space contains an absolutely convergent subsequence, so that this definition is sufficiently general to complete the space of step functions. Furthermore, every sequence of Lebesgue summable functions which converges in the metric of the space contains a subsequence which converges almost everywhere in the pointwise sense to the limit of the sequence. This definition selects in advance such subsequences.

In order to give meaning to the expression, "almost everywhere," it is necessary to define sets of measure zero. This may be done either in the usual way or in terms of absolute convergence. The two definitions, which are given below, can readily be proved equivalent.

DEFINITION 4. A set P of points is a *set of measure zero* if it can be covered by a finite or denumerable sequence of intervals of arbitrarily short total length.

DEFINITION 5. A set P of points is a *set of measure zero* if there exists an absolutely convergent series of step functions which is not absolutely convergent in the pointwise sense at any point of P.

DEFINITION 6. A function $s(x)$ is said to be *Lebesgue summable* if an absolutely convergent series of step functions $\sum_{n=1}^{\infty} u_n(x)$ exists such

that $s(x) = \sum_{n=1}^{\infty} u_n(x)$ at every point at which $\sum_{n=1}^{\infty} |u_n(x)|$ converges.

THEOREM 1. *If convergence for a series of step functions is taken as absolute convergence, conditions one through four are fulfilled.*

DEFINITION 7. $\int s(x)dx = \sum_{n=1}^{\infty} \int u_n(x)dx$, where $\sum_{n=1}^{\infty} u_n(x)$ is absolutely convergent and $s(x) = \sum_{n=1}^{\infty} u_n(x)$ at every point at which $\sum_{n=1}^{\infty} |u_n(x)|$ converges.

DEFINITION 8. $||s(x)|| = \lim_{k \to \infty} || \sum_{n=1}^{k} u_n(x)||$, where $||s(x)||$ is the *variation* of $\int s(x)dx$.

The class of sets which are measurable and of finite measure can be defined as those sets whose characteristic functions are summable and the measure of a set is the integral of its characteristic function.

The space L_p, $p > 1$, is constructed in the same way only using $||\mu||_p = [\int |\mu|^p dx]^{1/p}$ in place of $||\mu||$. For this space, it is necessary to restrict the domain of integration to a finite interval as the integral series does not in general converge over the infinite interval.

If the step functions are allowed to assume values in a Banach[3] space, the Bochner[5] integral is obtained.

The domain of the step functions may be a Boolean[4] ring of point sets, $A, B, C, \ldots$, for which an absolute value satisfying the following conditions is defined.

1. $|A| > 0$ if $A \neq 0$.
2. If $A = B$, then $|A| = |B|$.
3. $|A| = |A - AB| + |AB|$.
4. If $A = \sum_{i=1}^{\infty} A_i$, then $|A| = \lim_{n \to \infty} |\sum_{i=1}^{n} A_i|$.

The last condition does not imply the existence of infinite sums in the Boolean ring, it is merely the condition which must hold if there are any. Generalized in this way, the construction includes the Stieltjes integral.

We consider next the second type of extension. This extension leads to the Denjoy integral. The Denjoy integrable functions are known to be measurable and almost everywhere finite. The class of functions which are limit functions of series of step functions which are absolutely convergent in the pointwise sense almost everywhere is the class of measurable, almost everywhere finite functions. However, in general, the integral series for such series of step functions does not converge. In the case of the Lebesgue integral further restrictions were placed on the series of step functions considered in order to assure convergence of the integral series. The problem is to weaken these restrictions so as to be able to define the integral over a larger subclass of these functions. If, in addition to the condition of

pointwise absolute convergence almost everywhere, we require that the integral series be absolutely convergent, we again obtain the Lebesgue summable functions. Thus, to reach a larger class of functions, we must admit cases where the integral series is conditionally convergent. As the sum of a conditionally convergent series can be altered by changing the order of the terms, the problem resolves itself into finding criteria within the series itself for arranging it in a suitable order. These criteria are such as to permit iteration and a transfinite induction extending through the second class of ordinals.

We shall present this extension for the case of real valued functions defined over a finite interval of the linear continuum. It will be pointed out later that the generality of this method is comparable to that of the first method presented.

If $u(x)$ is a step function and I an interval, $u(x)$ on I will refer to the step function equal to $u(x)$ when x is on I and equal to zero when x is not on I. Similarly, if $s(x)$ is any function and E any set, $s(x)$ on E refers to the function equal to $s(x)$ on E and zero elsewhere. In what follows, $\sum_{n=1}^{\infty} u_n(x)$ is a series of step functions which is absolutely convergent in the pointwise sense almost everywhere and $s(x) = \sum_{n=1}^{\infty} u_n(x)$ at all points where $\sum_{n=1}^{\infty} |u_n(x)|$ converges. We are now in position to state the following theorem.

THEOREM 2. *In any finite interval I, a sequence of intervals I_n of arbitrarily small total length can be found such that $s(x)$ on $I - \sum_{n=1}^{\infty} I_n$ is Lebesgue summable (in fact, even continuous). Furthermore, an absolutely convergent series of step functions approaching $s(x)$ on $I - \sum_{n=1}^{\infty} I_n$ and zero elsewhere can be specified in terms of the sequences $u_n(x)$ and I_n even though $I - \sum_{n=1}^{\infty} I_n$ is not an interval.*

The Denjoy integral is now defined by means of the following induction.

DEFINITION 9. I. A function is integrable L^0 on an interval I if it is Riemann integrable on I.

II. A function $s(x)$ is integrable $L^{\xi+1}$ on an interval I if

(*a*) for every $\epsilon > 0$ a sequence of intervals $I_n \subset I$ can be found such that

(1) $\sum_{n=1}^{\infty} |I_n| < \epsilon$,

(2) $s(x)$ is not integrable L^{ξ} on any I_n,

(3) $s(x)$ is integrable L^{ξ} on $I - \sum_{n=1}^{\infty} I_n$;

(*b*) $\lim_{\longleftrightarrow 0} (L^\xi) \int_{I-\Sigma I_n} s(x)dx$ exists for all possible choices of sequences I_n, and, if these conditions hold, $L^{\xi+1} \int_I s(x)dx = \lim_{\longleftrightarrow 0} (L^\xi) \int_{I-\Sigma I_n} s(x)dx$.

III. If α is a limit ordinal, $s(x)$ is integrable L^α on I if $s(x)$ is integrable L^ξ for some $\xi < \alpha$ and $(L^\alpha) \int_I s(x)dx = (L^\xi) \int_I s(x)dx$.

Under this definition, the class of functions integrable L^1 is the class of Lebesgue summable functions and the induction can equally well be started at this point.

In view of Theorem 2, if the restriction *a* (2) were omitted in Definition 9, it would always be possible to find sequences I_n such that restrictions *a* (1) and *a* (3) would be fulfilled. In this case the induction breaks off after the first step and nothing is gained by further iteration. However, it offers an elegant approach to the Lebesgue theory. This approach was developed by Suckau[6] in his thesis.

The induction, as given in Definition 9, continues until Ω, the first ordinal of the third class, is reached. At this point it breaks off and further iteration gives nothing new. The integral L^Ω is the Denjoy integral. The Denjoy integral can be extended to infinite intervals in the usual way, by letting the end-point of a finite interval approach infinity.

This method of extension applies equally well in the more general case where the functions assume values in a Banach space and are defined over a normed Boolean ring.

[1] Riesz, F., *Acta Mathematica*, **42**, 191–205 (1920).
[2] Young, L. C., *The Theory of Integration*, Cambridge (1927).
[3] Banach, S., *Théorie des Opérations Linéaires*, Warsaw (1932), p. 53.
[4] MacNeille, H. M., these PROCEEDINGS, **24**, 188–193 (1938).
[5] Bochner, S., *Fundamenta Mathematicae*, **20**, 262–276 (1933).
[6] Suckau, J. W. T., *Amer. Jour. Math.*, **57**, 549–561 (1935).

ON IMPROPERLY IRREGULAR CYCLOTOMIC FIELDS

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If the so-called second factor[1] of the class number of the irregular algebraic field $k(\zeta)$; $\zeta = e^{2i\pi/l}$ is prime to the odd prime l, the field is called properly irregular, and improperly irregular if it is irregular and the second factor is divisible by l. In various papers[2] the writer has considered the first mentioned type, and a number of results were found concerning them. In connection with researches[3] on Fermat's Last Theorem it was found that all fields $k(\zeta)$ for every $l < 619$ were properly irregular and no improperly irregular cyclotomic fields have as yet been discovered.

We shall pursue the study of improperly irregular cyclotomic fields, however, in the hope that if none exist, we shall ultimately reach a contradiction.

In the consideration of fields of this type we shall find it convenient to separate them into classes and we shall say that the improperly irregular cyclotomic fields $k(\zeta)$ is of the first kind if there exists no unit η in $k(\zeta)$ such that

$$\eta = c \pmod{\lambda^{l-1}}; \qquad \lambda = 1 - \zeta. \tag{1}$$

The definition of this type of field indicates that it may have properties analogous to those of regular cyclotomic fields,[4] and we shall here show that this is indeed the case.

In what follows, any small italic letter (aside from s, which denotes a substitution) will denote a rational integer, and any Greek letter will denote an integer in the field $k(\zeta)$.

Pollaczek[5] showed that in any cyclotomic field whatever there exists a system of fundamental units η_i; $i = 1, 2, \ldots, l_1$; $l_1 = \frac{1}{2}(l-3)$ such that

$$\eta_i^{s-r^{2i}} = (\eta_i')^l \tag{2}$$

and we are using the Kronecker-Hilbert symbolic powers, s standing for the substitution (ζ/ζ^r), r being a primitive root of l. From this we have

$$\eta_i(x)^{s-r^{2i}} = (\eta_i'(x))^l + \frac{x^l - 1}{x - 1} A(x), \tag{3}$$

where x is an indeterminate and $A(x)$ is a polynomial with rational integral coefficients. Let $x = e^v$ in the above and denote in general

$$\left[\frac{d^k \log \omega(e^v)}{dv^k}\right]_{v=0} = 0$$

by $D_k(\omega)$, then (3) gives[6] for $k < l - 1$,

$$(r^k - r^{2i})D_k(\eta) \equiv 0 \pmod{l}$$

whence, the second factor in this relation $\equiv 0 \pmod{l}$ when $k \not\equiv 2i$. If $k = 2i$, then

$$D_{2i}(\zeta) \not\equiv 0 \pmod{l}, \tag{4}$$

for otherwise we note that from

$$\eta_i = a_i + b\lambda^s \pmod{\lambda^{s+1}} \tag{5}$$

we obtain, by replacing this with an equality involving e^v and using

$$D_{2a}(\eta) \equiv 0 \pmod{l}$$

for $a = 1, 2, \ldots, l_1$, that $s \geqq l - 1$; and this with (5) gives (1), which contradicts our hypothesis. Hence,

$$\eta_i \equiv a_i + b_i'\lambda^{2i} \pmod{\lambda^{2i+1}}$$

and

$$\eta_i^{l-1} \equiv 1 + b_i\lambda^{2i} \pmod{\lambda^{2i+1}} \tag{6}$$

with $b_i \not\equiv 0 \pmod{l}$. Now consider an integer prime to l in the included real field $k(\zeta + \zeta^{-1})$, call it ω and assume that $\omega \not\equiv c \pmod{\lambda^{l-1}}$. Then it is easy to show that

$$D_{2n+1}(\omega) \equiv 0 \pmod{l} \tag{7}$$

where $2n + 1 < l$. Hence,

$$\omega \equiv r_m + t_m\lambda^{2m} \pmod{\lambda^{2m+1}}, \tag{8}$$

where $2m < l - 1$. Now determine k_m so that

$$k_m b_m + t_m \equiv 0 \pmod{l} \tag{9}$$

which is always possible, since $(b_m, l) = 1$; then, using (6) for $i = m$, we have

$$\eta_m^{k_m(l-1)} \equiv 1 + kb_m\lambda^{2m} \pmod{\lambda^{2m+1}},$$

which with (8) gives

$$\eta_m^{k_m(l-1)} \equiv 1 + kb_m\lambda^{2m} \pmod{\lambda^{2m+1}} \tag{10}$$

or by (9) and (7)

$$\omega\eta_m^{k_m(l-1)} \equiv r_{m+1} + t_{m+1} + \lambda^{2m+2} \pmod{\lambda^{2m+3}}.$$

Now proceeding with the left-hand in the same way as was treated, we may multiply this by

$$\eta_{m+1}^{k_{m+1}(l-1)}$$

where $k_{m+1}b_{m+1} + t_{m+1} \equiv 0 \pmod{l}$; then we obtain

$$\omega\eta_m^{k_m(l-1)}\eta_{m+1}^{k_{m+1}(l-1)} \equiv r_{m+2} + t_{m+2}\lambda^{2m+4} \pmod{\lambda^{2m+5}}$$

so we see that finally

$$\omega\eta_m^{k_m} \ldots\ldots\ldots \eta_{l_1}^{k_{l_1}} \equiv a \pmod{\lambda^{l-1}}.$$

We assumed in (8) that $2m < l - 1$. For $2m \geqq l - 1$ we have

$$\omega \equiv r \pmod{\lambda^{l-1}}.$$

Hence, we have proved[7]

THEOREM I. *In an improperly irregular cyclotomic field $k(\zeta)$ of the first kind, any integer ω prime to l, belonging to the included real field $k(\zeta + \zeta^{-1})$, has the property that we may find a unit η in $k(\zeta)$ such that*

$$\omega\eta \equiv a \pmod{\lambda^{l-1}}, \tag{11}$$

where a is a rational integer.

In an improperly irregular cyclotomic field one of the units E_i, $i = 1, 2, \ldots, l$, is such that[8]

$$E_a = \sigma^l \tag{12}$$

where

$$E_a = \epsilon^R$$

where R is the symbolic power

$$1 + sr^{-2a} + s^2r^{-4a} + \ldots\ldots + s^{l_1}r^{-2al_1}$$

$$\epsilon = \left(\frac{(1-\zeta^r)(1-\zeta^{-r})}{(1-\zeta)(1-\zeta^{-1})}\right).$$

Suppose also that η_a in (2) is primary. If in an improperly irregular cyclotomic field an a exists satisfying (12) with also η_a primary, then we shall call such a field an improperly irregular cyclotomic field of the *second kind*. Supposing we have such a type of field, then since the η's are fundamental we have, using (12),

$$E_a^{l-1} = r_1^{c_1l(l-1)}\eta_2^{c_2l(l-1)} \ldots\ldots \eta_{l_1}^{c_{l_1}l(l-1)} \tag{13}$$

whence, somewhat as in another paper,[9] and using (3) and (4) we have

$$D_{2kl}(E_a) = c_k l D_{2kl}(r_k) \pmod{l^2}. \tag{14}$$

If $2a \not\equiv 2k$ and r is a primitive root of l^2, then[10] the left-hand member of the above congruence $\equiv 0$, (mod l^2). If $k = a$, then since η_a is primary,

$$D_{2al}(\eta_k) \equiv 0 \pmod{l}$$

and (13) gives

$$D_{2al}(E_a) \equiv 0 \pmod{l^2}$$

and[11]

$$B_{al} \equiv 0 \pmod{l^3}. \tag{15}$$

This result may be otherwise obtained by noting that if θ is any integer in $k(\zeta)$ with

$$\theta \equiv d + g\lambda^{l-2a} \pmod{\lambda^{l-2a+1}} \tag{16}$$

and $g \not\equiv 0 \pmod{l}$, $d \not\equiv 0 \pmod{l}$, and also that (13) gives[12]

$$E_a = \eta_a^{cl}$$

whence, since η is primary, we have

$$\sqrt[l]{E_a} \equiv \omega \pmod{\lambda^l}$$

then, since the left-hand member of this relation is therefore primary

$$\left(\frac{\sqrt[l]{E_a}}{\theta}\right) = 1$$

and by the law of reciprocity[13] we have

$$\frac{B_{al}}{al^2} D_{l-2a}(\theta) \equiv 0 \pmod{l} \tag{17}$$

and from (16) we have

$$D_{l-2a}(\theta) \not\equiv 0 \pmod{l}$$

whence (15).

We also have from a theorem of Pollaczek[14] that since η_a is primary, there is an integer ω_1, with

$$(\omega_1) = \mathfrak{a}^{l^n}$$

which is not the lth power of a principal ideal in $k(\zeta)$, and which is not primary. By another theorem[15] we have

$$\left(\frac{E_a}{\mathfrak{a}}\right) = \zeta^T = 1,$$

since E_a is an lth power, where

$$T \equiv \frac{r^{2a}-1}{2}(-1)^{\frac{s-1}{2}} B_{\frac{s+1}{2}} \frac{D_j(\omega)}{l^n} \pmod{l},$$

$$s = (2a-1)l^n,\ j = (l-2a)l^n,$$

$$\omega_1^{l-1} = \omega \equiv 1 \pmod{\lambda}.$$

The last factor in the expression for T, above, $\not\equiv 0 \pmod{l}$ by Pollaczek.[16] Hence,

$$B_{\frac{s+1}{2}} \equiv 0 \pmod{l^{n+1}}$$

and since $n > 0$, we note that

$$B_{\frac{s+1}{2}} \equiv 0 \pmod{l^2}. \tag{18}$$

But we also have[17]

$$B_{\frac{s+1}{2}} \equiv B_t \pmod{l^2}$$

where

$$t = \frac{(2a-1)l+1}{2};$$

hence, from (18)

$$B_t = 0 \pmod{l^2}; \tag{19}$$

now[18] if

$$\mu = \frac{l-1}{2}$$

$$B_s' = B_s/s$$

with x and y integers $\geqq 0$, then

$$xB_{a+y\mu}' \equiv xyB_{a+\mu}' - x(y-1)B_a' \pmod{l^2}, \tag{20}$$

also

$$yB_{a+x\mu}' \equiv xyB_{a+\mu}' - y(x-1)B_a' \pmod{l^2}, \tag{20a}$$

which for $y = 2a$, $x = 2a-1$ give, in view of (15) and (19), on subtraction

$$B_a' = 0 \pmod{l^2}$$

and this with (20) gives

$$B_{a+y\mu} \equiv 0 \pmod{l^2}$$

for any $y \geqq 0$. Whence,

THEOREM II. *If $k(\zeta)$ is an improperly irregular cyclotomic field of the second kind with $\zeta = e^{2i\pi/l}$*

$$E_a = \sigma l$$

with η_a as defined in (2), primary, then

$$B_{a+y\mu} \equiv 0 \pmod{l^2}$$

for y any integer $\geqq 0$.

We now apply this to the result[19] given without proof in an article on Fermat's Last Theorem. It was shown that the field $k(\zeta)$ defined by an odd prime l such that rational non-zero integers exist such that

$$x^l + y^l + z^l = 0$$

with x, y and z prime to each other and to l. In view of the latter relation it is then shown that a singular integer ω exists in $k(\zeta)$ which is not primary, and hence by Pollaczek's result there is a primary fundamental unit η_n such that

$$\eta^{s-r^{2n}} = \beta^l$$

where β is a unit in $k(\zeta)$, and also

$$E_n = y^l$$

for $2n = l - 3, l - 5, l - 7, l - 9, l - 11, l - 12$.

Hence, by Theorem II we prove the result in my *Bulletin* paper.

[1] Vandiver, these PROCEEDINGS, **16**, 743–749 (1930), with references to Kummer there given.

[2] These PROCEEDINGS, **15**, 202–207 (1929); *Monatshefte für Math. und Physik*, **48**, 369–380 (1939); these PROCEEDINGS, **25**, 586–591 (1939).

[3] Vandiver, *Trans. Amer. Math. Soc.*, **31**, 613–642 (1929); (we shall abbreviate this, henceforth, by T.); these PROCEEDINGS, **17**, 661–673 (1931); *Duke Math. Jour.*, **3**, 569–584 (1937), **5**, 418–427 (1939).

[4] Hilbert, *Werke*, Band I, pp. 278–289.

[5] Pollaczek, *Math. Zeits.*, **21**, 7 (1924).

[6] Vandiver, T., p. 619.

[7] Hilbert, loc. cit., p. 288.

[8] Vandiver, these PROCEEDINGS, **16**, 743 (1930).

[9] Vandiver, *Duke Math. Jour.*, **3**, 580 (1937).

[10] T., p. 620.

[11] T., p. 621.

[12] Vandiver, *Ann. Math.*, **31**, 124–125 (1930).

[13] Hasse, Bericht über neuere Untersuchungen, etc., *Jahresber. Deuts. Math. Verein*, 1930, p. 110.

[14] Loc. cit., pp. 21–22.

[15] Vandiver, T., **32**, 400 (1930).

[16] Loc. cit., pp. 19–22.

[17] Vandiver, *Ann. Math.*, **26**, 231 (1935).

[18] Pollaczek, loc. cit., p. 36; for interesting generalizations cf. Beeger, *Bull. Amer. Math. Soc.*, **44**, 688 (1938).

[19] Vandiver, *Bull. Amer. Math. Soc.*, **40**, 124 (1934).

A GENERAL THEORY OF SPECTRA. II

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In the present note, we shall discuss the theory of lattice-ordered abelian groups[1] and its relation to an earlier communication.[2] In particular, we derive the integration-free treatment of Riesz's operational calculus in a linear lattice,[3] which was previously promised.

We designate as an l-group any system L with elements $a, b, c, \ldots$ satisfying the composite condition

(1′) L is a system with two binary operations, $+$ and $*$, which (i) is an abelian group with respect to $+$; (ii) satisfies the commutative and associative laws with respect to $*$; (iii) satisfies the distributive law $a + (b * c) = (a + b) * (a + c)$ *for* $+$ *with respect to* $*$; (iv) satisfies the law of idempotence $a * a = a$.

The condition on L is equivalent to the requirement that L be a lattice-ordered abelian group: defining $a \leqq b$ if and only if $a*b = a$, we easily verify that $a * b$ is the greatest lower bound (or lattice-meet) of a and b. Hence the usual terminology of lattice theory may be applied to L without further comment: for instance, we call an element a positive or strictly positive according as $a \geqq 0$ or $a > 0$. The separate parts of (1′) closely resemble the postulates for commutative rings. Indeed, omitting (iv) and replacing (iii) by the distributive law $a * (b + c) = (a * b) + (a * c)$, we obtain the postulates in question. Moreover, retaining (iv) and replacing (iii) in the same way, we obtain the postulates for Boolean rings. Thus l-groups differ from rings primarily in a sort of inversion of the distributive law. Consequently the extensions, homomorphisms and representations for l-groups can be obtained by arguments not very different from those used in ring-theory.

Upon an l-group L we impose some, or all, of a series of conditions similar to those assumed in (I) for rings. These are:

(2′) for every a and every natural number n, the equation $nx = a$ has a solution in L;

(5′) for some element e, each a determines an associated natural number n such that $ne + a$ is positive;

(5″) for some element e, the relations $a \geqq 0$, $e * a = 0$ are equivalent to the relation $a = 0$;

(6′) if b is positive and if $b + na$ is positive for every natural number n, then a is positive;

(7) in terms of the distance $||a - b||$ (defined on the basis of (2′), (5′) and (6′) as the greatest lower bound of the rational numbers λ such that $|a - b| \leqq \lambda e$), L is a complete metric space;

(7′) if $\{a_n\}$ is a sequence of positive elements such that $a_n \geqq a_{n+1}$, then it has a greatest lower bound.

In (5′) and in (5″), the special element e must be strictly positive; and any e satisfying (5′) also satisfies (5″). An l-group with the property (7′) is said to be complete. A complete l-group necessarily has property (6′); and a complete l-group satisfying (2′) and (5′) also satisfies (7). In order that an l-group satisfy (2′), (5′), (6′) and (7), it is necessary and sufficient that L be a Banach lattice containing a special element e such that the relations $||a|| \leqq 1$ and $|a| \leqq e$ are equivalent.

Any l-group L can be extended to an l-group L^* (consisting of the "formal fractions" a/n) which has the property (2′) and also any of the properties (5′), (5″) (6′) possessed by L. Similarly any l-group with the properties (2′), (5′), (6′) can be extended (by metric completion) to an l-group satisfying (2′), (5′), (6′) and (7). Thus any complete l-group L satisfying (5′) can be extended to an l-group L^* satisfying (2′), (5′), (6′) and (7); and we shall subsequently see that L^* also has property (7′).

The homomorphisms of an l-group are studied by means of its normal sub-l-groups, which we here designate as l-ideals since their rôle is analogous to that of ideals in ring-theory. A non-void subclass $\mathfrak{a}$ of an l-group L is thus said to be an l-ideal if and only if, whenever $a_1, \ldots, a_p$ are in $\mathfrak{a}$ and $|b| \leqq |a_1| + \ldots + |a_p|$, then b also is in $\mathfrak{a}$. An l-ideal $\mathfrak{a}$ is said to be prime if $|a_1| * |a_2| \in \mathfrak{a}$ implies $a_1 \in \mathfrak{a}$ or $a_2 \in \mathfrak{a}$; an l-ideal $\mathfrak{a}$ is prime if and only if the quotient-l-group $L/\mathfrak{a}$ is simply ordered. Associated with an arbitrary non-void subclass $\mathfrak{a}$ of an l-group are the least l-ideal $\mathfrak{a}^*$ containing $\mathfrak{a}$, and the l-ideal $\mathfrak{a}'$ of all elements b such that $|b| * |a| = 0$ for every a in $\mathfrak{a}$; for them the relations $\mathfrak{a} \subset \mathfrak{a}^* \subset \mathfrak{a}''$, $\mathfrak{a}' = \mathfrak{a}'''$, $\mathfrak{a}'^* = \mathfrak{a}^{*\prime} = \mathfrak{a}'$ are readily verified. Moreover, $\mathfrak{a}^*$ is easily identified as the class of all elements b such that $|b| \leqq |a_1| + \ldots + |a_p|$ for some elements $a_1, \ldots, a_p$ in $\mathfrak{a}$. In a complete l-group, the l-ideals $\mathfrak{a}^*$ and $\mathfrak{a}'$ are complete l-groups.[4]

The analysis of complete l-groups can easily be reduced to the study of complete l-groups satisfying (5′), as we shall now show. If e is any strictly positive element in a complete l-group L, then L is the direct sum

of the l-ideals $\{e\}'$ and $\{e\}''$ and the l-ideal $\{e\}''$ is essentially determined by the l-ideal $\{e\}^*$: for, if a is any positive element in L, then $a' = \inf\{a - (ne * a)\} \epsilon \{e\}'$, $a'' = a - a' = \sup\{ne * a\} \epsilon \{e\}''$, and $ne * a \epsilon \{e\}^*$. We thus have a homomorphic mapping $L \to L/\{e\}' = \{e\}''$ *which preserves least upper and greatest lower bounds (for sequences).* Not only are the l-groups $\{e\}''$ and $\{e\}^*$ both complete, but they also satisfy the respective conditions (5″) and (5′) with e as the required special element in each case. If e is now allowed to range over the class of all strictly positive elements in L (or merely over a class of element e_α such that $\alpha \neq \beta$ implies $e_\alpha * e_\beta = 0$ while $e_\alpha * |a| = 0$ for every α implies $a = 0$), then no element a other than 0 is mapped by *every* homomorphism $L \to \{e\}''$ into the zero element of $\{e\}''$. Applying the principle of McCoy and Montgomery,[5] and observing that the direct sum of complete l-groups satisfying (5″) is an l-group of the same kind, we obtain

THEOREM 1. *A complete l-group can be isomorphically imbedded, with preservation of least upper and greatest lower bounds (for sequences), as a sub-l-group in a direct sum of complete l-groups satisfying (5″)—and hence as a sub-l-group in a complete l-group satisfying (5″).*

Of course, in a complete l-group L satisfying (5″), we have $L = \{e\}''$ for the special element e of (5″) and are thus left to study the complete l-group $\{e\}^*$, which satisfies (5′).

Shifting our attention next to the case of an l-group L with properties (2′), (5′), (6′) and (7), we derive for it a function-theoretic representation due to Kakutani;[6] this representation is analogous to the representation for rings given in (I). An argument applied by Krull[7] in the case of commutative rings enables us to construct a maximal l-ideal omitting e. This l-ideal is necessarily metrically closed and prime; and the simply ordered quotient-l-group $L/\mathfrak{a}$ is easily identified with the l-group of real numbers (with their natural ordering). In particular, if a is an arbitrary element in L, the l-ideal $\{||a||e - |a|\}^*$ cannot contain e; and we may therefore determine $\mathfrak{a}$ so that it contains $||a||e - |a|$. The map $L \to L/\mathfrak{a}$ then takes $|a|$ into the real number $||a||$. Applying the principle of McCoy and Montgomery, together with topological complements first formulated in explicit terms by Kakutani,[8] we obtain

THEOREM 2 (Kakutani's Theorem). *An l-group L satisfying (2′), (5′), (6′) and (7) is isomorphic to the l-group of ALL continuous real functions on a uniquely determined bicompact Hausdorff space $S(L)$; the positive elements in L are represented by precisely those functions with no negative values and the element e by the constant function 1.*

From (I) we immediately have the

COROLLARY. *In order that L be complete it is necessary and sufficient that $S(L)$ be a Boolean space which is associated with a completely additive Boolean algebra.*

Thus in any complete l-group satisfying (2′) and (5′) we may set up an operational calculus in the manner indicated in our earlier note (I), thereby obtaining, without use of integration, results similar to those of Riesz.[8]

Recalling now that any complete l-group L satisfying (5′) can be imbedded in an l-group L^* satisfying (2′), (5′), (6′) and (7), we can at once represent L isomorphically as a sub-l-group L_0 of the l-group of continuous functions on $S(L^*)$. By virtue of the explicit construction of L^* in terms of L, we see that every continuous function on $S(L^*)$ can be uniformly approximated by rational linear combinations of functions in L_0. Coupling this topological fact[9] with the fact that L_0 is complete, we can show that $S(L^*)$ is the representative Boolean space for a completely additive Boolean algebra, and *hence that L^* is a complete l-group*. Now at any point p in $S(L^*)$ the values assumed by functions in L_0 constitute a sub-l-group $L_0(p)$ of the real numbers. Since this l-group may be discrete, consisting of numbers of the form $\pm k/n$ where n is fixed and $k = 0, 1, 2, 3, \ldots$, we are led to consider the set $S_N(L)$ of all points p where the least strictly positive number in $L_0(p)$ is of the form k/N. We put $T_N(L) = S_1'(L)S_2'(L) \ldots S_{N-1}'(L)S_N(L)$. Further investigation of a topological nature then yields

THEOREM 3.[10] *If L is a complete l-group satisfying (5′), then there exist a uniquely determined bicompact Boolean space $S(L)$, which represents a completely additive Boolean algebra A, and a uniquely determined sequence $\{S_N(L)\}$ of closed sets in $S(L)$, where $S_M(L)S_N(L) = S_P(L)$ with P the g. c. d. of M and N and where the complementary open sets $S_N'(L)$ represent completely additive ideals in A, such that L is isomorphic to the l-group L_0 of all those continuous real functions on $S(L)$ which assume on each set $T_N(L)$ no values other than $\pm k/N$, $k = 0, 1, 2, \ldots$, $N = 1, 2, 3, \ldots$; and if the point p belongs to no set S_N, then $L_0(p)$ is the l-group of real numbers. Conversely, if S and S_N are prescribed arbitrarily subject to the conditions indicated, then there exists a complete l-group L satisfying (5′) and the special relations $S(L) = S$, $S_N(L) = S_N$ for $N = 1, 2, 3, \ldots$.*

We shall not formulate theorems describing the representations for complete l-groups satisfying (5″) and for unrestricted complete l-groups, since the details are rather complicated. However, we can easily see in a general way what representations are possible. In treating a complete l-group L satisfying (5″), we first apply Theorem 3 to obtain a representation of $\{e\}^*$, where e is the special element given in (5″). We then use this representation to furnish representatives for those elements which are in $L = \{e\}''$ but not in $\{e\}^*$. By virtue of earlier remarks, it is easily verified that each such element has an essentially unique representative function F with the following properties: (α) F is finite and continuous except at the points of an associated closed nowhere dense set; (β), if n is any natural number, there exists a neighborhood of the associated exceptional set such that F assumes thereon no value between $-n$ and n; (γ) on

each set $T_N(\{e\}^*)$, the function F assumes no finite values other than those of the form $\pm k/N$. In calculating sums and lattice-meets of such functions, one must obviously neglect nowhere dense sets. With this proviso, there is no difficulty in showing that the combined aggregate of all the continuous functions and all the (non-bounded) functions with properties (α) and (β) above is actually a complete l-group satisfying (5″); and that L can be isomorphically imbedded therein as a sub-l-group L_0. Clearly L_0 must satisfy the condition (γ) but *is not in general characterized thereby*. Combining this result in judicious fashion with Theorem 1, we arrive finally at a function-theoretic representation for an arbitrary complete l-group.

Finally, we remark that the close parallel between the ring-representation given in (I) and the l-group-representation given by Kakutani's Theorem is one which extends even to the validating arguments. If R is any ring with the properties required in (I) we may treat it also as an l-group satisfying (2′), (5′), (6′) and (7), by virtue of the definition $a * b = {}^1/_2(a + b) - {}^1/_2\sqrt{(a-b)^2}$. A *closed* non-void subclass of R is an ideal if and only if it is an l-ideal. Hence if the prime l-ideals $\mathfrak{a}$ used above in proving Kakutani's Theorem are interpreted for R, they are found to be closed divisorless ideals; and the associated homomorphisms $R \to R/\mathfrak{a}$ map R on the real number field. Conversely, the technique of a direct discussion of R yields also a proof of Kakutani's Theorem. Thus, apart from the preliminary construction of square roots in R, the arguments used in establishing these representations for rings and for l-groups are essentially identical.

[1] For general lattice-theory and its technical terminology, we refer to Garrett Birkhoff, *Lattice Theory*, New York, 1940.

[2] M. H. Stone, *Proc. Nat. Acad. Sci.*, **26**, 280–283 (1940). This paper will be cited here as (I).

[3] F. Riesz, *Ann. Math.* (2), **41**, 174–206 (1940).

[4] Riesz has discussed the systems $\mathfrak{a}^*$ and $\mathfrak{a}'$ at some length in ref. 3, above.

[5] See N. H. McCoy and Deane Montgomery, *Duke Math. Jour.*, **3**, 455–459 (1937).

[6] See S. Kakutani, *Proc. Imp. Acad. Tokyo*, **16**, 63–67 (1940). The proof sketched here differs from the one communicated to me orally by Dr. Kakutani last October; it involves some technical simplifications, if I understand the situation correctly. See also the final paragraph of this paper.

[7] See W. Krull, *Math. Annalen*, **101**, 729–744 (1929), especially page 732.

[8] See Kakutani, ref. 6, above.

[9] See M. H. Stone, *Trans. Am. Math. Soc.*, **41**, 375–481 (1937), especially Theorem 82, the proof of which is essentially lattice-theoretical.

[10] I understand that J. v. Neumann has also derived results in close contact with those stated in Theorem 3 and those sketched in the next paragraph—particularly as regards the "splitting off" of the discrete l-groups $L_0(p)$ and the calculus modulo nowhere dense sets met in the next paragraph. The precise nature of v. Neumann's results and the extent to which their proofs are connected with observations made in (I) are both unknown to me.

GENERAL INVARIANTS OF IRREGULAR ANALYTIC ELEMENTS

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1. Statement of the Problem.—In this paper, we shall give a summary of the results obtained in the study of the invariant theory of analytic arcs or elements in the geometry of the plane based on the infinite group G of arbitrary point transformations. Our results are valid for the group of real point transformations of the real plane; or for the group of complex point transformations of the complex plane. Kasner has developed the corresponding theory in the conformal geometry of the complex plane.[1]

The configuration we shall consider is an analytic arc (regular or irregular) together with a specific point of the arc. This compound object we shall term an *analytic element.* It consists of a point—the base point which shall be taken as the origin throughout this paper, and an analytic arc through that point. It may be described also as a *differential element of infinite order.*[2]

The most general analytic element is represented by writing x and y as integral power series in a parameter t without constant terms. If the parameter t is eliminated, then y is found as a series in x which may proceed according to integral or fractional powers of x. If fractional exponents enter and cannot be avoided by interchanging x and y (this will then necessarily be the case for *any* choice of rectangular axes), we shall call the element *irregular;* otherwise the element is *regular.*

Our new problem is to classify with respect to the group G of arbitrary regular point transformations all analytic elements regular and irregular.

Any regular element is obviously equivalent to any other and may be changed to the canonical form $y = 0$. But for irregular elements, the results are complicated. For example, the cuspidal element $y = x^{3/2}$ cannot be converted into the irregular element $y = x^{4/3}$. For these curves differ qualitatively in the nature of the singular point at the origin. However, our main result is that, in general, *irregular elements have absolute differential invariants.* Certain exceptions arise, namely, those in which the corresponding series in x proceeds according to powers $\geqq 3$ of the square root, powers $\geqq 4$ of the cube root and three other types in which the corresponding series in x proceeds according to powers of the fourth root of x. All the other cases possess absolute differential invariants.

2. Statement of the General Results.—We shall assume throughout this paper that our group G of arbitary point transformations leaves invariant

the fixed point o (the origin) of our analytic element and that it is regular in the neighborhood of o. Thus our group G is

$$X = (\alpha_{01}x + \alpha_{02}x^2 + \ldots) + y(\alpha_{10} + \alpha_{11}x + \ldots) + y^2(\alpha_{20} + \alpha_{21}x + \ldots) + \ldots,$$

$$Y = (\beta_{01}x + \beta_{02}x^2 + \ldots) + y(\beta_{10} + \beta_{11}x + \ldots) + y^2(\beta_{20} + \beta_{21}x + \ldots) + \ldots, \quad (1)$$

where the jacobian $J = \alpha_{01}\beta_{10} - \alpha_{10}\beta_{01} \neq 0$.

Any analytic element may be defined by setting x and y equal to two power series in a parameter t without constant terms. Let the positive integer $p \geqq 1$ be the minimum of the two exponents of the leading terms of the power series in t; we may assume this to appear in the x series. Therefore our analytic element may be written in the form

$$y = c_p x^{p/p} + c_{2p}x^{2p/p} + \ldots + c_{rp}x^{rp/p} + c_q x^{q/p} + c_{(q+1)}x^{(q+1)/p} + \ldots, \quad (2)$$

where the positive integer $q = rp + s$ is such that the positive integers r and s satisfy the inequalities $r \geqq 1$ and $o < s < p$.

If $c_q = 0$ and if thereafter every coefficient of an actual fractional power of x is zero, our element is *regular*. Otherwise if $c_q \neq 0$, it is *irregular*.

For an irregular element, the integer p is called the *index* and the integer $q = rp + s$ is called the *rank*. All irregular elements obtained by taking arbitrary values of the coefficients, but fixing the values of the integers p and q, we shall define as forming the *single species* (p, q). It is easy to show that both the index p and the rank q are arithmetic invariants under the group G of arbitrary point transformations.

Absolute differential invariants, that is functions of the coefficients unaltered by the group G of arbitrary point transformations, exist for all irregular species (p, q) *except in the cases* $(4, 5)$, $(4, 6)$, $(4, 7)$, $(3, q)$, *and* $(2, q)$. *The species* $(4, 5)$ *may be divided into two distinct sets, and the species* $(4, 7)$ *may be separated into three distinct sets, the members of any one of these sets being equivalent. The species* $(4, 6)$ *and* $(3, q)$ *may be separated into a denumerably infinite number of such distinct sets. All the elements of the species* $(2, q)$ *are equivalent—the canonical form is* $y = x^{q/2}$. *Finally all the regular elements are transformable into each other, the standard form being* $y = 0$.

In the remainder of the paper, we shall give the absolute differential invariants of lowest order for each species.

3. *The Absolute Differential Invariants of Lowest Order.*—In this section, we state the following fundamental results:

THEOREM 1. *The species* $(p, q = rp + s)$ *for which* $r \geqq 2$ *and* $o < s < p - 2$, *or for which* $r = 1$ *and* $2 < s < p - 2$ *possesses the following absolute differential invariant of lowest order*

$$\frac{c_q c_{q+2}}{c_{q+1}^2}. \tag{3}$$

The order of our invariant is $q + 2$, its weight is $2q + 2$, and its degree is 2.

THEOREM 2. *The species* $(p, q = rp + s)$ *for which* $r \geqq 2$ *and* $1 < s = p - 2$, *or for which* $r = 1$ *and* $3 < s = p - 2$ *possesses the absolute invariant*

$$\frac{c_q^2 c_{q+3}}{c_{q+1}^3}. \tag{4}$$

The order is $q + 3$, weight is $3q + 3$, and degree is 3.

THEOREM 3. *The species* $(p, q = rp + s)$ *for which* $r \geqq 2$ *and* $2 < s = p - 1$, *or for which* $r = 1$ *and* $3 < s = p - 1$ *possesses the absolute invariant*

$$\frac{c_q c_{q+3}^2}{c_{q+2}^3}. \tag{5}$$

The order is $q + 3$, weight is $3q + 6$, and degree is 3.

THEOREM 4. *The species* $(p, q = p + 1)$ *for which* $p > 4$ *possesses the absolute invariant*

$$\frac{[(p+1)^2 c_{p+1}^2 c_{p+4} - 2(p+1)(p+2)c_{p+1}c_{p+2}c_{p+3} + {}^1/_3(p+2)(3p+5)c_{p+2}^3]^2}{[(p+1)c_{p+1}c_{p+3} - {}^1/_2(2p+3)c_{p+2}^2]^3} \tag{6}$$

The order is $q + 3 = p + 4$, weight is $3q + 3 = 3p + 6$, and degree is 6.

THEOREM 5. *The species* $(p, q = p + 2)$ *for which* $p > 5$ *possesses the absolute invariant*

$$\frac{c_{p+2}[(p+2)c_{p+2}c_{p+5} - (2p+5)c_{p+3}c_{p+4}]}{c_{p+3}^3}. \tag{7}$$

The order is $q + 3 = p + 5$, weight is $3q + 3 = 3p + 9$, and degree is 3.

THEOREM 6. *The species* $(5, 7)$ *possess the absolute invariant*

$$\frac{c_7(c_7^2 c_{11} - 8c_7 c_9^2 - 8c_8^2 c_9)}{c_8^4}. \tag{8}$$

The order is 11, weight is 32 and degree is 4.

THEOREM 7. *The species* $(5, 8)$ *possesses the absolute invariant*

$$\frac{c_8^2(8c_8c_{12} - 9c_9c_{11})}{c_9^4}. \tag{9}$$

The order is 12, weight is 36 and degree is 4.

THEOREM 8. *The species (4, 5) possesses only the following relative differential invariant*

$$(10c_5c_7 - 11c_6^2)^2/c_5^5. \tag{10}$$

Our species may be subdivided into two distinct sets according as this relative invariant is or is not zero. The normal forms of these two sets are respectively $y = x^{5/4}$ *and* $y = x^{5/4} + x^{7/4}$.

THEOREM 9. *The species (4, 6) possesses the positive odd integer* $Q \geqq 7$, *the first odd power of the fourth root of x which appears in the series expansion for y, as an arithmetic invariant and the expression*

$$c_Q^5/c_6^{Q-1}, \tag{11}$$

(which cannot vanish) as the only relative differential invariant. The canonical form is $y = x^{3/2} + x^{Q/4}$.

THEOREM 10. *The species (4, 7) possesses the expression*

$$c_9^3/c_7^4, \tag{12}$$

as a relative differential invariant. If this is zero, then the species (4, 7) possesses the additional relative invariant

$$(14c_7c_{13} - 17c_{10}^2)/c_7^3. \tag{13}$$

There are no more relative invariants. Thus the species (4, 7) may be divided into three distinct sets according as (a) both $c_9 = 0$ *and* $14c_7c_{13} - 17c_{10}^2 = 0$; *(b)* $c_9 = 0$ *but* $14c_7c_{13} - 17c_{10}^2 \neq 0$; *and (c)* $c_9 \neq 0$. *The normal forms of these three distinct sets are respectively (a)* $y = x^{7/4}$; *(b)* $y = x^{7/4} + x^{13/4}$; *and (c)* $y = x^{7/4} + x^{9/4}$.

THEOREM 11. *The species (3, q) may be separated into the following two distinct sets: (a) The set for which there exists a first Qth power of the cube root of x such that* $q < Q < 2q - 3$ *and* $q + Q \equiv 0 \bmod 3$; *and (b) the set for which no such Q exists. The set (a) of this species possesses only the relative differential invariant (which cannot vanish)*

$$c_Q^{q-1}/c_q^{Q-1}. \tag{14}$$

The standard forms of these two sets are respectively (a) $y = x^{q/3} + x^{Q/3}$; *and (b)* $y = x^{q/3}$.

THEOREM 12. *The elements of the species (2, q) are all equivalent. The canonical form of any such element is* $y = x^{q/2}$.

4. *The Table Exhibiting the Order of Our Differential Invariants.*—The following table exhibits some of the results in detail.

	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
2	*		*		*		*		*		*		*		*	
3		*	*		†	†		†	†		†	†		†	†	
4			†	‡	†		11		14		15	17	18		19	21
5				9	11	12	12		13	14	16	17		18	19	21
6					10	11	11	13	14		15	16	17	19	20	
7						11	12	12	13	15	16		17	18	19	20
8							12	13	13	14	15	17	18		19	20
9								13	14	14	15	16	17	19	20	
10									14	15	15	16	17	18	19	21

Here the species is determined by the value of p in the left column and the value of q in the top row. The blank spaces denote the fact that there are no such species. In the body of the table we find the order of the absolute differential invariants discussed above. The asterisk indicates that the corresponding species has no absolute invariant and further that all members of that species are equivalent under the group G of arbitrary point transformations. The dagger indicates that there are no absolute differential invariants but that the members are not all equivalent: there exists in some cases a certain relative differential invariant, hence there is a division of such a species into a finite number of subspecies, all distinct with respect to the group G of arbitrary point transformations. The double dagger indicates that there is a certain arithmetic invariant so that the species (4, 6) may be divided into an infinite member of subspecies.

We may compare and contrast this table for *general* point transformations with the corresponding table for the *conformal* theory given on page 339 of the paper cited in the first reference. The asterisk * is more prevalent in the present table, and so is the dagger †, while the double dagger ‡ is a new feature; and the orders of the invariants are higher (as is to be expected).

The present paper serves as an introduction to a new aspect of *restricted topology* [see abstracts in *Bull. Amer. Math. Soc.* (1927)].

[1] Kasner, "Conformal Classification of Analytic Arcs or Elements: Poincare's Local Problem of Conformal Geometry," *Trans. Amer. Math. Soc.* **16**, 333–349, July (1915).

[2] Kasner has introduced elsewhere the concept of divergent differential element of infinite order: This corresponds to a divergent power series and may be represented by a non-analytic arc having specified values for all the successive derivatives. Thus to every power series corresponds a geometric entity which may be real or imaginary, regular or irregular, convergent or divergent. This entity is the most general differential element. If it is convergent we call it an analytic element or more loosely, an analytic arc or curve.